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# Canadian Journal of Botany

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## EPIDEMIOLOGY STUDIES ON THE SEPTORIA DISEASE OF OATS[1]


R. V. CLARK AND F. J. ZILLINSKY



### Abstract

Ascospores were found to be the principal cause of primary infection in the disease of oats caused by *Leptosphaeria avenaria* f. sp. *avenaria*. Macrospores produced in the leaf lesions on oat plants were responsible for the secondary spread of the fungus and the subsequent development of the black stem phase of the disease. Viable ascospores were present throughout the growing season and provided the means for widespread development of the disease. The purpose of the microspores is not clearly understood but they would appear to be of little importance in the initiation and subsequent spread of the disease. Seed-borne infection was found to be of no importance in the epidemiology of this disease.


### Introduction

The disease of oats caused by *Leptosphaeria avenaria* Weber f. sp. *avenaria* (imperfect stage, *Septoria avenae* Frank f. sp. *avenae*) is of major importance in Eastern Canada. This fungus infects the leaves, stems, glumes, and kernels of the oat plant. Symptoms appear first on the leaves as small purplish or dark spots developing into irregular greyish-brown lesions on leaves and leaf sheaths. Lesions develop on leaf sheaths at about heading time and from there the fungus invades the culm tissue resulting in the development of the black stem phase of the disease. Usually this phase is the most destructive form of the disease as the oat crop may suffer from severe lodging due to the weakening of the stem tissue. However, leaf lesions are very prevalent each year and may reduce the yield potential considerably. Infection of the seed occurs occasionally causing conspicuous blackened areas on the lemma and palea.

This fungus produces macrospores, microspores, and ascospores under natural conditions (3, 7). The role of these three spore forms in the overwintering and subsequent spread of the disease as primary inoculum is not completely understood. Weber (10) found that pycnidia and macrospores in diseased leaf tissue remained viable for at least 6 months when stored under field conditions during the winter. Dickson (2) stated that this fungus persists from one season to the next as mycelium and pycnidia on crop residue. In Iowa, Huffman (3) reported that the fungus persists through the late summer and winter in the mycelial form on the stubble. He observed that microspores were present in early April and were abundant during May and June. By the use of spore traps, he found that the microspores were wind-borne and


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concluded that they constituted the primary inoculum. Shaw (7) found that microspores, as well as macrospores, were present on mature plants from Eastern Canada at the end of August. The microspores remained abundant on the nodes and internodes throughout the winter and spring. Macrospores and ascospores were also found to be present in the spring. By late May and early June all three spore forms were present in the field and at that time the number of microspores appeared to be decreasing, the macrospores increasing, and the ascospores very abundant. She came to no conclusion as to which form constituted the primary inoculum.

There have been conflicting reports on the importance of infected seed as a possible source of primary inoculum. This type of infection was reported by Stanton (9) to be very prevalent in the North Central United States in 1952. Simons and Murphy (8) could find no evidence of seedling infection from the seed. Lund and Shands (4) obtained seedling infection from infected seed grown in the greenhouse and found that such infection was influenced by moisture and temperature. However, they felt that the seedling infection was in no way responsible for the spread of the disease to mature plants. Seed treatment eliminated much of the seedling infection. Noble and Montgomerie (6) stated that this disease is seed-borne in Scotland. The first symptoms in the field take the form of fine black necrotic streaks on the base of the coleoptile and could very easily be overlooked if the seedlings were not examined closely. They were not able to determine the importance of this early seedling infection in spreading the disease to the more mature plants. They found that some control of the seedling infection could be obtained by seed treatment, especially if the fungus was fairly superficial (5).

Experiments were carried out to determine if seed-borne infection is important in initiating primary infection; to determine if the elimination of inoculum in the immediate area of the oat plants reduces primary infection; and to ascertain the significance of ascospores in the initiation and subsequent development of the disease. The results of these studies under field and greenhouse conditions are reported in this paper.

## Procedures and Results

### *Seed-borne Infection*

The effect of seed treatment on disease development in the field was investigated by treating naturally infected seed of three oat varieties with two chemical seed dressings. Ceresan M-2X (ethylmercury *p*-toluene sulphonanilide) and Canuck Seed Guard (phenolmercury acetate and ethylmercury chloride) were the seed dressings used and the latter was employed in an experiment set up at two different locations. The concentrations of the chemicals used were those suggested by the manufacturers for seed-borne cereal diseases. The seed treatments used did not reduce disease development or improve the emergence of the seedlings. Furthermore, no evidence of seedling infection could be found in the check plots. By the end of the growing season there was a very heavy infection of this disease on all plots. Apparently the diseased kernels were not important in initiating this infection.

As a further check on the possibility of seed transmission, several tests were conducted in the greenhouse. It was felt that specific levels of moisture and temperature might be required for the seedling infection to develop and these might not be present at the time the field tests were tried. Oat seedlings were grown in greenhouse flats placed in chambers in which various moisture levels up to a completely saturated atmosphere were used at temperatures ranging from 65° F to 80° F. Included in each test was seed of two varieties obtained from Eastern Canada showing severe symptoms, seed from Eastern Canada showing no symptoms, and seed from Western Canada from a location where this disease does not occur. In some cases, the seed was planted in greenhouse soil while in other cases it was planted in sterile sand. The seedlings were grown to the second or third leaf stage and carefully examined during this time for infection. In no case was there any evidence of seedling infection due to this fungus and, for a short period of time after emergence, the seedlings from the infected seed appeared to be more vigorous than those from the two controls.

*Soil Inoculum*

A field test was set up in an effort to determine if the primary inoculum was confined in its spread to the immediate area around the oat plants or if the inoculum could originate from a source some distance away. It was felt that the application of chemicals with fungicidal properties to the soil in the immediate area of the plants would eliminate inoculum from this source. In this experiment three chemicals, Dowfume MC-2 (methyl bromide), Vapam (sodium methyldithiocarbamate), and Anti-carie (hexachlorobenzene) were applied to the soil in plots measuring approximately 20 ft × 8 ft. Clover had been grown in this area the previous year and the soil was cultivated in the spring and then treated prior to seeding. The chemicals were applied, where possible, according to the recommendations of the manufacturers. Two varieties of oats were seeded in each of the plots and four replicates were used. During the growing season, the plants in these plots were carefully examined and compared with those grown in control plots for time of initial infection and subsequent disease development by *L. avenaria* f. sp. *avenaria*. It was found that typical symptoms appeared in the treated plots just as early as in the control plots and were just as prevalent. This pattern was consistent throughout the growing season. Yield data showed that the plants from the treated plots yielded no more than the plants from the control plots.

*Ascospores*

Both the macrospores and microspores are exuded from their fruiting structures in the form of cirri or gelatinous matrices which on drying become hard. These spores require water for dispersal and, as Shaw (7) has pointed out, it would seem unlikely that they would be disseminated by air currents. On the other hand, the ascospores are forcibly discharged from the ascus and are ideally suited to wind dispersal. Ascospores are produced in great abundance during the growing season on the previous year's straw and stubble. These factors suggest that the ascospores are the major source of primary

inoculum. Several experiments were carried out to determine their importance in this respect.

Collections of overwintered straw and stubble have been made for the past 2 years during the spring and summer months. To determine the presence of ascospores, portions of the straw showing black stem symptoms were placed in moist chambers and suspended over glass slides to catch any spores that might be released. In both years it was found that mature asci and ascospores were present on the straw during the first week of June but not before that time. Ascospores were obtained from material collected earlier but several periods of wetting and drying of the straw were required before the ascospores were released and presumably they matured while in the moisture chambers. During the first week of June, the ascospores were deposited on the slides 6 to 12 hours after being placed in the moist chambers. By the third week in June the greatest number of ascospores were being released from the straw and the time required in the moist chamber was the least (3 hours). This peak period of release lasted for approximately two weeks and following this the length of time required gradually increased and the number of spores deposited gradually decreased. However, it was found that ascospores were present on the straw and stubble as late as September and even then they were quite plentiful. The ascospores from all samples examined were capable of germinating and most of those within a given sample had germinated after approximately six hours in the moist chamber. When the samples of straw and stubble were examined carefully, microspores and occasionally macrospores could be found. However, in no case was either spore type found deposited on the slides. Both of these types were found to be prevalent in the spring when ascospores were not present but were not so prevalent later when the ascospores became mature.

Spore traps were employed to determine if the ascospores were present in the air under field conditions. These were placed in various locations including areas that had been in oats, hay, and summer fallow the previous year. Considerable difficulty was encountered in reading the slides due to the presence of dust, insects, and other extraneous material. Because of this the slides could only be exposed for a short period, especially if the spore traps were located on cultivated areas. Mature ascospores were found occasionally on the slides but not regularly. The ascospores were found most frequently on the spore traps placed in the areas planted to oats the previous year. As a further check on the presence of ascospores in the air, chambers made up of wire frames covered with polyethylene, approximately 15 in. × 24 in. × 24 in. in size, were placed over areas in the field where the previous year's oat stubble was present. These frames were kept as air-tight as possible and this provided a high humidity inside. A small table-like device 12 in. high was placed inside the frame and several clean slides were laid on top of the table. Six hours later these slides were removed and ascospores were present on every slide examined. Apparently a high humidity is required for the release of the ascospores from the asci on the straw but once released they have no difficulty being spread by air currents.

The ability of the ascospores to cause infection was investigated in the greenhouse and under field conditions. Ascospores from many of the samples of the overwintered straw and stubble collected were used to inoculate oat plants in the greenhouse. This was done by suspending pieces of the straw over water agar in Petri dishes. Later germinating ascospores plus a little agar were carefully picked off and these were spread on the surface of oat leaves in an area close to the junction of the leaf blade and leaf sheath. The inoculated area was tagged for later identification and the plants were placed in a moisture chamber for 48 hours and finally placed on a greenhouse bench. Checks were included with agar only spread on the leaves. Symptoms developed on most of the infected leaves 48 hours following inoculation and after 1 week typical lesions were observed. Ten to 14 days from the time of inoculation, the lesions were examined and it was found that pycnidia had developed with typical macrospores present. Some of the infected plants were left on the greenhouse bench for a month or more and by that time the infection had spread down the leaves and leaf sheaths to the stems and typical black stem symptoms were apparent. Disease symptoms did not develop on the check plants. Apparently the macrospores serve as the main source of inoculum for the secondary spread of this fungus. In several instances, macropycnidia were taken from the lesions that originated from ascospore infection and put on water agar. When the macrospores were exuded from the pycnidia, small pieces of agar were picked off and oat plants were inoculated as described previously. Infection was obtained in every case, resulting ultimately in the production of typical black stem symptoms.

During June of 1958, samples of overwintered straw and stubble were collected from eight different areas across Eastern Canada. Ascospores were obtained from these straw and stubble samples as described previously and used to inoculate oat plants in the greenhouse. Three plants of Victory oats were grown per pot and at least two leaves of two of the plants were inoculated for each sample. The third plant was used as a control. After inoculation the plants were kept in a moist chamber for 48 hours and then placed on a greenhouse bench. The number of infected leaves was recorded 1 week after in-

TABLE I

Disease development on oat plants grown in the greenhouse and inoculated with ascospores of *L. avenaria* f. sp. *avenaria* obtained from straw collected from various locations in Eastern Canada

| Origin of straw | No. of leaves inoculated | No. of leaves infected | Macrospores produced | Black stem symptoms |
|---|---|---|---|---|
| Ottawa No. 1 | 4 | 4 | Yes | Yes |
| Ottawa No. 2 | 4 | 4 | Yes | Yes |
| L'Assumption | 5 | 0 | No | No |
| Ste. Anne de la Pocatière | 4 | 0 | No | No |
| Lennoxville | 4 | 4 | Yes | Yes |
| Fredericton | 4 | 3 | Yes | Yes |
| Nappan | 4 | 3 | Yes | Yes |
| Charlottetown | 4 | 2 | Yes | Yes |
| Hunter River | 4 | 3 | Yes | Yes |

oculation. Two weeks later the leaves were examined for the presence of pycnidia and macrospores and 6 weeks after inoculation the plants were examined for the presence of black stem symptoms. The results of this test are presented in Table I. Seven of the nine samples of ascospores tested were capable of causing infection and 23 of the 37 leaves inoculated became infected. In every case following infection the development of the fungus continued and pycnidia and macrospores were produced in the lesions and eventually typical black stem infection developed. The reason for the failure of two of the samples of ascospores to cause infection could not be explained.

In the field experiments, overwintered oat straw containing ascospores was used to inoculate Lanark oats. The polyethylene-covered chambers described previously were used as moisture chambers and a small amount of infected stubble from the previous year's oat crop was suspended from the top of these chambers. The inside of the chambers and the straw were sprayed lightly with water to raise the humidity and then the chambers were placed over oat plants. At least 30 plants were enclosed by each chamber. The chambers were left over the plants for 2 days and then removed. These same chambers and straw were used to inoculate plants at two later dates. Four chambers, including one containing no straw, were used each time. On the last date of inoculation an additional series was included in which the plants were sprayed only with macrospores which had been grown on oat-leaf agar (1). Five random plants from each of the treated areas were collected and were used to determine the average number of lesions per leaf. Leaves from uncovered plants in neighboring areas were also taken to determine the extent of natural infection. The data obtained are presented in Table II.

These data indicate that the ascospores from overwintered straw were quite capable of causing infection under field conditions. The first lesions appeared approximately seven days after inoculation and 1 to 2 weeks later the lesions were plentiful. Disease development on the plants from the chamber in which no straw was placed was approximately the same as on the plants from the untreated areas. However, there was a marked difference in the amount of disease development between the inoculated and non-inoculated plants.

TABLE II

Infection on Lanark oats inoculated in the field (*a*) with overwintered oat straw containing ascospores of *L. avenaria* f. sp. *avenaria* and (*b*) with macrospores of the same fungus

| Date inoculated | Date examined | Average number of lesions per leaf | | | | |
|---|---|---|---|---|---|---|
| | | Chamber 1 | Chamber 2 | Chamber 3 | Chamber 4 (no straw) | Uncovered area |
| June 13 (*a*) | June 23 | 25 | 15 | 45 | 0 | 0.5 |
| | July 4 | 65 | 34 | 72 | 3.5 | 4 |
| June 16 (*a*) | June 23 | 4 | 3 | 5 | 0 | 0.5 |
| | July 4 | 85 | 70 | 95 | 2.5 | 5 |
| June 24 (*a*) | July 4 | 43 | 32 | 57 | 5.5 | 5.5 |
| June 24 (*b*) | July 4 | 56 | 74 | | 5 | 5 |

(*a*) The same straw and chambers were used for all three inoculation dates.
(*b*) Inoculated with macrospores and agar only.

A comparison was made between the lesions from the treated and untreated plants and it was found that their over-all development was very similar. This indicated that disease development on the untreated plants was also initiated by ascospores. Apparently ascospores were released from the overwintered straw over a considerable period of time because disease development was just as severe after the third time the straw was used as it was after the first time, 11 days earlier. As well, the concentration of ascospores released was dependent upon the amount of overwintered straw placed in the chambers. Chamber 3 consistently gave the highest number of lesions per leaf and chamber 2 the lowest number. The use of macrospores as inoculum did not result in a greater number of disease lesions but the disease appeared to develop faster with the use of this inoculum. This no doubt was due to the fact that a high concentration of macrospores was involved in each infection court whereas with the ascospores the initial infection was probably started by a single spore. Furthermore, when macrospores were used as inoculum, large brown necrotic areas quickly developed on the leaves rather than the small pin-point purple-brown lesions which first developed after infection with the ascospores. The same brown necrotic lesions developed some seven to ten days after the plants were infected with ascospores.

A date of seeding experiment was set up in 1958 to determine the effect of early to late planting on disease development by this fungus. Several varieties of oats were planted at 2-week intervals starting on May 1 with three replicates planted at each date. Notes on disease development were recorded several times during the growing season. Small pin-point purple-brown lesions were first observed on the plants in the earliest date of seeding during the third week of June. This coincided quite well with the first release of ascospores, described previously. Mature ascospores were first found in the field during the first week in June, about ten days to two weeks before symptoms appeared on the oat plants. Similar lesions developed on the plants in the second date of seeding about a week later than in the first date of seeding. This same pattern was consistent throughout the growing season. If the material in the different dates of seeding was compared at one time during the growing season then those seeded early showed the greatest disease development. However, the disease development in the material seeded later eventually became as severe as that seeded early. Similar small purple-brown lesions were observed during August and September on material planted in late July and August. This agreed with the earlier observation that ascospores occurred under natural conditions in the field as late as September.

## Discussion

There are several possible sources of primary inoculum for infection of oats by *Leptosphaeria avenaria* f. sp. *avenaria*. The various spore forms which develop from overwintered mycelium or fruiting structures on the straw or stubble, i.e., macrospores, microspores, and ascospores, could serve as the primary inoculum (3, 7). In addition, the seed-borne infection in the oat

kernels could provide a means of initiating the disease in the spring (4, 6). The present investigations indicate that ascospores constitute the major source of primary inoculum.

The application of fungicides to soil in the immediate vicinity of the plants was not effective in reducing infection. However, it was noted that both Dowfume M-2X and Vapam were very effective in reducing the weed population, indicating that sufficient chemical had been applied to the soil. Furthermore it was observed that most of the initial lesions on the young seedlings occurred on the upper leaves, indicating inoculum had fallen from above. Thus it appeared that the inoculum was air-borne and capable of being carried some distance.

Ascospores infect oat plants readily under both field and greenhouse conditions. Mature ascospores were found in the field during the first week of June, which agrees with the observations of Huffman (3) and Shaw (7). Approximately ten days to two weeks later the first symptoms developed and by the end of June lesions were plentiful. Just prior to this time the maximum number of ascospores were released from the overwintered straw and at this same time only a short period of high humidity was required for the release of the ascospores. However, ascospores were released from the straw over a period extending from June to September and by planting oats at different times during this period it was found that purple-brown pin-point lesions typical of ascospore infection were produced throughout the growing season. On the other hand, as Shaw (7) observed, microspores were plentiful during April and May but decreased in numbers in June. Macrospores were found only occasionally on the overwintered straw.

The means of dispersal of the different spore forms would also appear to favor the ascospores as the major source of primary inoculum. The ascospores are ejected from the asci and thus are capable of being spread by the wind. By the use of spore traps and also slides exposed in moisture chambers, it was found that the ascospores were present in the air under field conditions. No effort was made to determine how far the ascospores could be carried by the wind and still remain viable. The ascospores are thin-walled and presumably could not survive in the air for long periods of time. In Eastern Canada oat fields are small and numerous and often oats are planted 2 years in succession in the same field. Thus the ascospores would only need to be wind-borne for short periods of time before coming in contact with a susceptible host. Both the macrospores and microspores are slime spores and are released from fruiting structures in the presence of water. When these fruiting structures are kept in a humid atmosphere the spores are exuded as cirri which on drying become hard and require further moisture to soften them. Shaw (7) used wind currents up to 20 m.p.h. to determine if the macrospore cirri and macrospores could be dislodged and spread by this means. She found that no cirri or macrospores were deposited on plates held perpendicular and immediately behind the leaves but that in other tests rust and powdery mildew spores were deposited readily under these conditions. Presumably the

microspores and microspore cirri would act in a similar manner to macrospores although Huffman (3) using spore traps reported that the microspores were wind-borne and disseminated in great numbers. In the present investigations, microspores were not found on the slides with the spore traps used, although if they were present in small numbers they may have been overlooked. Considerable extraneous material was deposited on the slides which made examination difficult. When the overwintered straw was suspended over slides in Petri dishes, heavy concentrations of ascospores were deposited on the slides. However, there were no microspores or macrospores found on these slides even though both types of spores could be found on the straw used.

The initial symptoms produced from infection by the different spore forms may be determined by the conditions existing during inoculation. Shaw (7) reported that the symptoms produced from infection by ascospores, macrospores, and microspores were identical. Presumably this was under artificial conditions in the greenhouse where the spores were used in high concentrations and suspended in water. With this type of infection large irregular blotches are produced on the leaves. When the ascospores were allowed to fall on the leaves from the overwintered straw above, small individual purple-brown pin-point lesions developed. This same type of lesion was found to be general during the early infection under natural conditions. Eventually these lesions developed to the point where they coalesced and large blotches were produced. The small lesions were, no doubt, initiated by one or, at the most, a few spores. Since ascospores are dispersed in the air, they would appear to be the only spore that would be spread individually. Shaw (7) suggested that cirri might be dislodged by insects and then spread by the wind. However, because of the concentration of spores in a cirrus, a large blotch would quickly develop once conditions became favorable for infection. Splashing rain, as suggested by Huffman (3), might spread macrospores and microspores but the concentration of spores in the drops of water could be quite high so again large blotches would develop. This would be an important means of secondary spread of the fungus.

The length of time required for the germination of the different spore forms could be a factor in determining which type serves as the primary inoculum. Ascospores germinated within 2 to 6 hours on glass slides in humidity chambers and on water agar. Macrospores usually require a slightly longer period than the ascospores but microspores have to be kept in a humid atmosphere for 48 hours before any appreciable number of spores germinate (7). Infection of oats in the greenhouse can be obtained with ascospores and macrospores after 24 hours in a moist chamber. With microspores, on the other hand, no infection is noticeable until after a period of 96 to 120 hours under high moisture conditions. Thus a period of at least 4 to 5 days of high humidity would be required to obtain infection of oats in the field with microspores. Periods of continuous wet weather of this duration occur only rarely. Therefore, it would appear unlikely that microspores are the major source of the early and continuing infection that occurs in the field.

Infection of oat seedlings from seed-borne inoculum has been shown to occur (4, 6). However, its importance as primary inoculum has not been established. The experiments conducted in this investigation have failed to show any evidence of this type of infection. However, it is possible that under different environmental conditions infection from this source of inoculum might be of some consequence.

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# THE PATHOGENICITY OF ISOLATES OF SEPTORIA AVENAE F. SP. AVENAE[1]

R. V. Clark and F. L. Drayton

## Abstract

All isolates of *Septoria avenae* Frank f. sp. *avenae* studied were pathogenic on selected varieties of oats and there was no evidence of a differential reaction. The virulence of the isolates varied greatly and this variability was inherent in all groups of isolates. The dimensions, septations, and numbers of macrospores produced by the isolates on oat-leaf agar also varied greatly but there was no correlation between these variable characters and the disease rating of the isolates.

## Introduction

The fungus *Leptosphaeria avenaria* Weber f. sp. *avenaria* (imperfect stage *Septoria avenae* Frank f. sp. *avenae*) causes *Septoria* leaf blotch and black stem, a serious disease in many of the oat-growing areas of the world. Previous to 1950 this disease was practically unknown in Eastern Canada, but since then its prevalence has increased and now it is recognized as a major oat disease in Ontario, Quebec, and the Atlantic provinces.

Three types of fruiting structures are produced during the life cycle of the fungus (6). Macropycnidia and macrospores become plentiful in lesions on the leaves and leaf sheaths during the growing season. Micropycnidia and microspores appear when the plants near maturity and are abundant on stubble and straw in the fall and spring. Mature asci and ascospores of the perfect stage become abundant on the previous year's stubble by early summer. Macrospores are usually produced in culture, but microspores may develop if the cultures are kept at a low temperature. The production of the perfect stage in culture has been reported only by Weber (7).

Johnson (5) and Hooker (3) found that the cultural characteristics of different isolates of this fungus varied greatly. Hooker (3) was able to obtain fairly uniform cultural types after several successive single spore transfers but these often reverted to the variable condition when stored for a period of time. In pathogenicity tests, he found that the virulence of the stabilized cultures derived initially from a single macrospore or from a germ-tube tip could vary considerably, ranging from lower to higher than the original. Experiments were initiated at Ottawa to obtain more comprehensive information on the pathogenic variability of the fungus. This information is important in determining the uniformity of the reaction of oat varieties to this fungus under natural and artificial conditions. This paper summarizes the results of greenhouse studies on the extent of the pathogenic variability of isolates of *S. avenae* f. sp. *avenae* obtained from various sources in Canada and the United States from 1955 to 1957.

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## Materials and Methods

Isolations were usually made from spore suspensions prepared by picking off a few pycnidia from infected leaves and leaf sheaths and placing them in 1 cc of water. The resulting suspension of spores was flooded on water agar which was then incubated at room temperature for 4 to 6 hours. Single germinating spores were picked off and transferred to oat-leaf agar slants (4) using five to eight slants per sample. The monopycnidial isolates originated from single pycnidia. With some of the microspore isolates, single hyphal tips instead of spores were transferred to the agar slants. Three to 4 weeks after isolation the best sporulating culture was selected from each group of slants and kept as the representative isolate. The isolates were transferred then to oat-leaf agar plates by means of a spore suspension and following three to four transfers at weekly intervals on this medium the isolates were used to inoculate oat seedlings in the greenhouse. In agreement with the observations of Hooker (3), to keep the isolates in a sporulating condition, it was necessary to make frequent transfers with spores on oat-leaf agar. Inoculum for the greenhouse tests was prepared by mixing each oat-leaf agar culture with 100 ml of water in a Waring blender. A small amount of the wetting agent Tween 20 was added to the inoculum to obtain maximum coverage of the oat foliage. All isolates were tested at least twice.

At first the isolates were tested in the greenhouse on four varieties of oats, Abegweit, Lanark, Rodney, and C.D. 4090 (Wales),[2] and later two more varieties C.D. 5924 and 5055-46 (Ottawa hybrid selection) were added to the group. These varieties were selected because of their field reaction to the fungus which ranged from very susceptible (C.D. 5924 and Lanark) to tolerant (5055-46 and C.D. 4090). The oats were grown in soil in waxed paper cups placed in greenhouse flats. Four replicates were included in each test. The plants were inoculated in the third to fourth leaf stage. Prior to inoculation they were kept for 12 hours in chambers maintained at 100% relative humidity at a temperature of 70° F. The inoculum was sprayed on the plants with a pressure-vacuum sprayer at approximately 10 pounds pressure. Following a 48-hour incubation period in the inoculation chambers, the plants were returned to the greenhouse bench. Disease notes were recorded 7 to 8 days after inoculation. Ratings were made on the basis of estimates of the percentages of the leaf area affected. The fungus developed little on plant tissues that were produced subsequently to inoculation, therefore ratings were taken only on the tissues that were actually inoculated.

## Results

The first collection of isolates studied included a group of 10 from single microspores, a group of 10 from single macrospores, and a group of 6 from single macropycnidia. The diseased material from which these isolates were obtained was grown at several locations in Eastern Canada. These isolates were tested on the four oat varieties Abegweit, Lanark, Rodney, and C.D.

[2]C.D. refers to cereal crops accession number.

4090. Before the inoculum of each isolate was used to infect the oat seedlings, it was examined microscopically to determine the concentration of macrospores per milliliter of suspension. Each isolate was then given a rating according to the concentration of spores with zero representing no spores and 5 a heavy concentration of spores. The average disease and spore concentration ratings of these isolates are shown in Fig. 1.

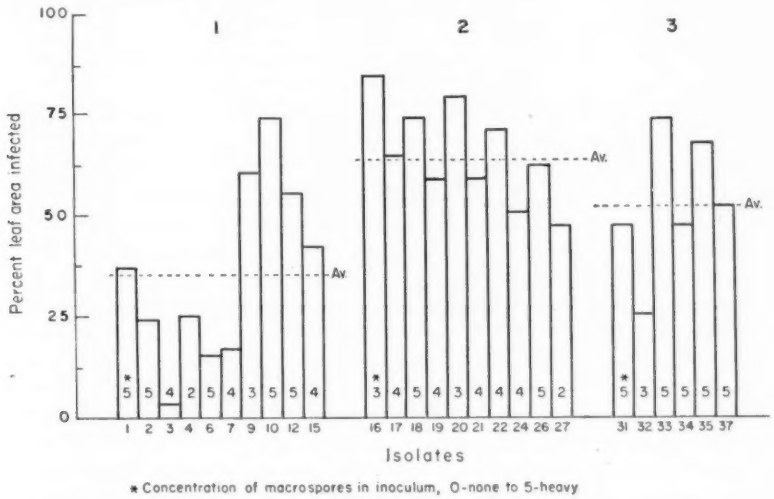


FIG. 1. The average virulence of isolates of *Septoria avenae* f. sp. *avenae* on four varieties of oats. 1. Monomicrospore isolates. 2. Monomacrospore isolates. 3. Monomacropycnidial isolates.

The second collection of isolates included three groups of single macrospore isolates obtained from different sources. The first group of isolates was from different locations across Canada. However, most of these isolates came from Ontario, Quebec, and the Maritime Provinces. The diseased samples from which this group was obtained were taken from the same oat variety (Rodney) where possible. The second group of isolates was taken from different oat varieties grown at Fredericton, New Brunswick, and the third group from a single lesion on a leaf grown at Ste. Anne de la Pocatière, Quebec. The isolates included in the second collection were tested on six varieties including the four used with the first collection plus C.D. 5924 and 5055-46. The inoculum of each isolate was examined as described previously prior to inoculation. The average disease and spore concentration ratings of this collection of isolates are shown in Fig. 2.

Five isolates[3] were obtained in 1957 from different locations in the North Central United States, and tested in the greenhouse on the six oat varieties.

[3]Secured through the courtesy of Dr. A. L. Hooker, formerly of the Department of Plant Pathology, University of Wisconsin, Madison. Present address: Department of Plant Pathology, University of Illinois.

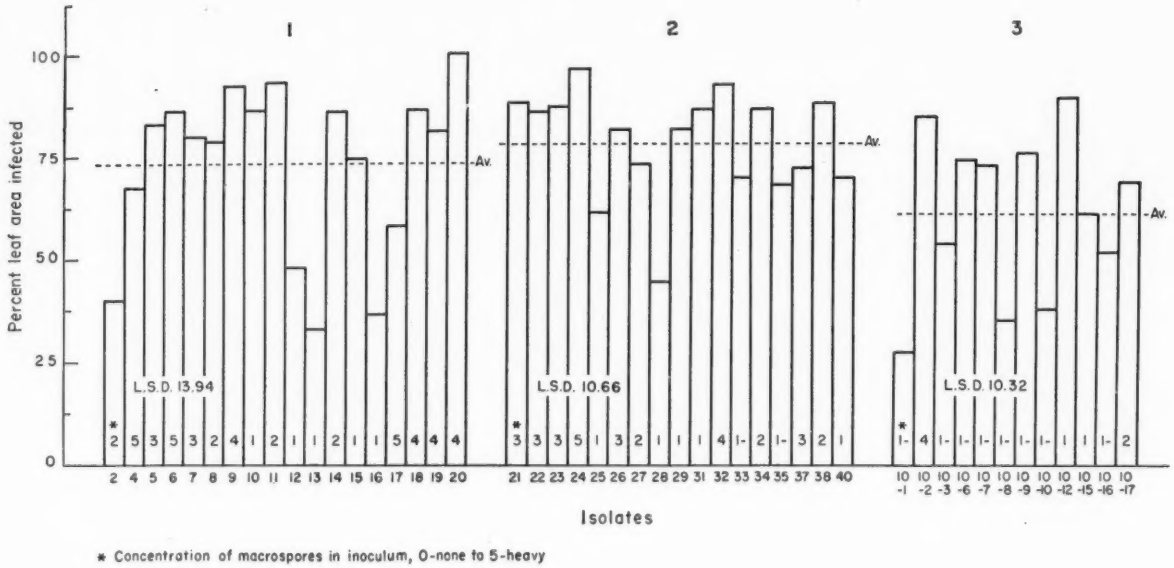


FIG. 2. The average virulence of isolates of *Septoria avenae* f. sp. *avenae* on six varieties of oats. 1. Monomacrospore isolates from several locations across Canada. 2. Monomacrospore isolates from Fredericton, N.B., obtained from different varieties of oats. 3. Monomacrospore isolates obtained from a single lesion (obtained from the same sample as isolate 10).

TABLE I

The average virulence of isolates obtained from the North Central United States on six varieties of oats

| Isolate | Concentration of spores* | Per cent leaf area infected |
|---|---|---|
| 5 (*H. avenae*) | — | 87.9 |
| 11 | 2 | 71.3 |
| 12 | 4 | 72.9 |
| 18-2 | 5+ | 75.0 |
| 19-2 | 4 | 83.7 |

*Concentration of macrospores in inoculum: 0—none to 5—heavy.

One of these isolates (isolate 5), identified as *Helminthosporium avenae* Eidam., was included for comparison with the *Septoria* isolates. The *Septoria* isolates originated from single macrospores and were treated in the same manner as those tested previously. The average disease and spore concentration ratings of these isolates are presented in Table I.

All isolates studied were pathogenic on the test varieties but they varied greatly in virulence. An analysis of variance of the disease ratings obtained with the second collection of isolates showed that the difference in virulence among isolates was highly significant (Fig. 2). The variability in virulence was not restricted to any group of isolates. A similar variability was present among the isolates from a single lesion, as well as among the single pycnidial isolates. The isolates from different varieties grown at one location as a group showed the highest average disease rating and the single microspore isolates showed the lowest average disease rating. The greatest range in virulence was present in the group of isolates obtained from different locations across Canada, and in the group of single microspore isolates. The smallest range in virulence was apparent in the isolates from the North Central United States and in the group of single macrospore isolates. Isolate 5 (*H. avenae*, Table I) was similar in virulence to highly virulent *Septoria* isolates. This isolate was of interest in the greenhouse tests because of the close similarity in symptom expression to those of *S. avenae* f. sp. *avenae*. It was almost impossible to separate this isolate from the others when the type of damage and lesions produced on the test varieties were compared. Furthermore, the test varieties responded in the same order with this isolate as with the other isolates. A comparison of the damage caused by two isolates of *S. avenae* f. sp. *avenae* is shown in Fig. 3: 1, a highly virulent isolate; and 2, a weakly virulent isolate.

As noted previously, it is difficult to keep the fungus in a sporulating condition. Nevertheless, the numbers of macrospores produced by the isolates on oat-leaf agar was also very variable. All the isolates were treated in approximately the same way and yet the sporulation ranged from very heavy to very poor. There was no consistent association between the concentration of macrospores in the inoculum of an isolate and the subsequent disease rating. A high concentration of macrospores in the inoculum did not always result in a high disease rating. Poorly sporulating isolates usually produced much more mycelium than abundantly sporulating isolates. However, some poorly

sporulating isolates produced high disease ratings. With these isolates, the mycelium was just as effective as macrospores in initiating infection. Most of the isolates in the group obtained from a single lesion showed a low ability to produce macrospores on agar. These isolates were obtained from the same diseased sample as was isolate 10 (Fig. 2, group 1) which also produced few spores. However, one of these isolates (10-2) showed a high capacity for spore production. Even though the isolates were obtained from a very limited area this character, as well as the disease ratings, was quite variable.

The test varieties of oats did not show a differential response to the isolates. This was confirmed statistically by analysis of variance of the data from the second collection of isolates. However, there was a significant difference in the average reaction of the six test varieties within two of the three groups of isolates in this same collection, but these differences were not large. The reaction of the six oat varieties to a selected group of isolates obtained from different locations across Canada (Fig. 2, group 1) is shown in Table II. These data show that if isolates were highly virulent on one variety, then they were highly virulent on the other five; and if they were weakly virulent on one variety, then they were weakly virulent on the other five. The varieties fell into two groups according to their reaction to the isolates: quite susceptible—Lanark, Rodney, and C.D. 5924; those with some tolerance—Abegweit, 5055-46, and C.D. 4090. A similar reaction was obtained with all other groups of isolates.

TABLE II

The reaction of six varieties of oats to a selected group of single macrospore isolates obtained from different locations across Canada (Fig. 2, group 1)

| | Per cent leaf area infected | | | | | | |
|---|---|---|---|---|---|---|---|
| Isolate | Abegweit | 5055-46 | C.D. 4090 | C.D. 5924 | Rodney | Lanark | Average |
| 2 | 35 | 35 | 25 | 35 | 55 | 52.5 | 39.6 |
| 4 | 60 | 55 | 57.5 | 75 | 70 | 72.5 | 66.7 |
| 9 | 70 | 85 | 90 | 97.5 | 100 | 100 | 90.4 |
| 10 | 77.5 | 77.5 | 85 | 87.5 | 90 | 90 | 84.6 |
| 11 | 85 | 85 | 92.5 | 97.5 | 92.5 | 95 | 91.3 |
| 13 | 27.5 | 32.5 | 30 | 35 | 32.5 | 37.5 | 32.5 |
| 15 | 65 | 65 | 75 | 77.5 | 75 | 80 | 73.7 |
| 16 | 40 | 55 | 45 | 50 | 47.5 | 52.5 | 48.3 |
| 19 | 67.5 | 70 | 80 | 77.5 | 80 | 82.5 | 77.9 |
| 20 | 92.5 | 97.5 | 97.5 | 97.5 | 100 | 100 | 97.5 |
| Average | 62.0 | 65.7 | 67.7 | 73.0 | 74.2 | 76.2 | |

When this study was first begun, it was noted that the macrospores produced by the isolates varied considerably in length, width, and number of septa. It appeared that the macrospores of certain isolates were more variable than those from others. A test was made to determine if this variability in spore morphology was associated with macrospore production and virulence. Samples of the spore suspensions prepared for each isolate in the first collection of isolates studied were retained and microscope mounts were prepared for detailed examination of the macrospores. A total of 25 macrospores were

PLATE I

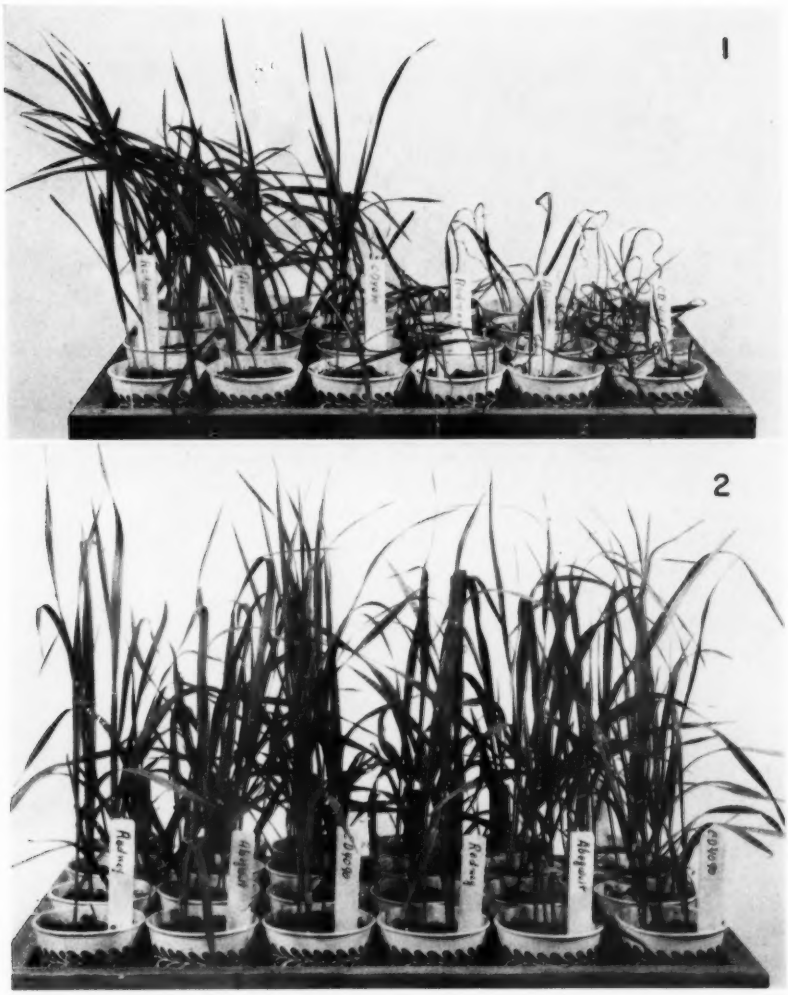


FIG. 3. A comparison of the damage caused by two single macrospore isolates of *Septoria avenae* f. sp. *avenae* on three varieties of oats. 1. A highly virulent isolate (isolate 24, Fig. 2, group 2). 2. A weakly virulent isolate (isolate 16, Fig. 2, group 1). From left to right in each print, three control varieties and the same three varieties inoculated.

TABLE III

Some morphological characters of samples of macrospores obtained from each monomacrospore isolate (Fig. 1, group 2)

| Isolate | Length ($\mu$) | | | Width ($\mu$) | | | Number of septa | | |
|---|---|---|---|---|---|---|---|---|---|
| | Min. | Max. | Av.* | Min. | Max. | Av.* | Min. | Max. | Av.* |
| 16 | 27.0 | 48.6 | 38.9 | 2.7 | 4.5 | 3.6 | 2 | 7 | 3.8 |
| 17 | 23.4 | 44.1 | 34.5 | 3.2 | 4.5 | 3.9 | 2 | 3 | 2.9 |
| 18 | 27.0 | 50.4 | 37.7 | 2.7 | 4.5 | 3.8 | 2 | 4 | 3.0 |
| 19 | 27.5 | 47.7 | 36.8 | 2.7 | 4.9 | 4.2 | 1 | 3 | 2.8 |
| 20 | 30.6 | 45.5 | 37.1 | 3.2 | 4.5 | 3.8 | 2 | 5 | 2.9 |
| 21 | 22.5 | 53.1 | 40.3 | 3.2 | 4.5 | 3.8 | 3 | 14 | 8.1 |
| 22 | 23.4 | 50.4 | 37.7 | 2.7 | 4.5 | 3.5 | 1 | 5 | 2.9 |
| 24 | 29.3 | 42.8 | 36.3 | 3.2 | 4.5 | 3.9 | 3 | 7 | 3.7 |
| 26 | 19.7 | 41.4 | 33.2 | 2.7 | 4.5 | 3.8 | 2 | 8 | 4.1 |
| 27 | 32.4 | 59.4 | 42.0 | 2.7 | 4.1 | 3.2 | 3 | 5 | 3.3 |

*Average of 25 spores.

examined at random for each isolate. The minimum, maximum, and average spore length, width, and number of septa of the samples of macrospores from the single macrospore isolates (Fig. 1, group 2) are shown in Table III. Fairly large differences in macrospore measurements were apparent among the isolates. There was an even greater difference among isolates in the numbers of septa per spore. Some isolates were quite uniform in this regard while others varied a great deal—up to a maximum of 14 septa per macrospore (isolate 21, Table III). Similar results were obtained with the single microspore and macropycnidial isolates. There appeared to be no association of the variability in morphological characters and the variability in macrospore production and virulence. Uniform and variable isolates could not be correlated with either high or low spore production or high or low virulence.

## Discussion

All the isolates studied were pathogenic and there was no differential reaction of the test varieties. Race differences were not demonstrated. This fungus is known to be quite variable in other characters (3, 5). Because of this extensive variability, the establishment of specific races might be quite difficult. The test varieties used in this study were chosen because of differences in the severity of infection under field conditions, employing the widest range in varietal reaction then available. However, only varieties of *Avena sativa* L. (common oats) were included. The use of a group of test varieties with a broader genetical background might have given a wider range in reaction and made possible the differentiation of races. It has been reported by Derick (1) that varieties from other species, particularly among the diploid chromosome group, have greater resistance to this fungus.

A possible explanation of the variability of the isolates in spore production on agar and in virulence might be that the isolates differed in genetic make-up for these characteristics. However, there was not necessarily any association between the factors for spore production and virulence. In his study on the

cultural variability of this fungus, Hooker (3) found that a separation of factors for pathogenicity as well as for cultural stability occurred, as subcultures derived from successive single-spore transfers differed significantly in pathogenicity.

In the groups of isolates studied, the most extensive variability would be expected in the group of single macropycnidial isolates and the least in the single macrospore isolates obtained form a single lesion on a leaf. However, all groups of isolates showed a wide variability in the various characters. It is of interest that the isolates from the North Central United States and Western Canada reacted similarly on the test varieties to those from Eastern Canada. It would appear that disease development by this fungus is basically the same in widely separated areas of the country.

*S. avenae* f. sp. *avenae* was originally described by Frank (2) in 1894 as having macrospores 28–43 $\mu$ long, 3.5 $\mu$ wide, and with 2–4 septa. The results of this study show that these dimensions are too limited for the macrospores of this fungus, especially when grown on a medium such as oat-leaf agar.

## Acknowledgments

The authors wish to thank Angela McBride for technical assistance and A. A. Scott for preparing the charts and photographs.

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# WHEY AS AN INHIBITOR OF STRIPE MOSAIC OF BARLEY[1]

W. A. F. HAGBORG[2] AND W. S. CHELACK[2]

## Abstract

The results of experimental applications of whole milk and whey to stripe mosaic virus inoculum or to barley plants prior to inoculation in the greenhouse established that contact transmission of stripe mosaic of barley was strongly inhibited by both milk and whey. Similarly, both skim milk and whey reduced the spread of field infection when applied as a spray. The low cost of whey suggests its possible economic utilization in the commercial control of virus diseases of plants.

## Introduction

Interest in the control of contact transmission of the tobacco mosaic disease by the use of milk (1, 2, 3, 4, 5, 7, 11, 12) was recently revived by Crowley (6) and Hare and Lucas (9), who obtained abundant evidence that whole milk could reduce the amount of contact transmission in the tobacco mosaic disease. Little similar work has been done with the barley stripe mosaic (BSM) disease, although Slykhuis (13) obtained inhibition of infection with milk as well as with the juices of several plant species by additions to the infective juice used as inoculum. The present paper presents evidence for a sharp interference by whey with the contact transmission of BSM.

The efficacy of whey as a research material for the control of virus diseases of plants appears to be especially significant. According to Harvey and Hill (10) tens of millions of gallons of whey are discarded annually.

## Materials and Methods

The degree of inhibition of infection of BSM was indicated by a reduction in the proportion of infected plants and/or a reduction in the severity of infection. Where a comparison was made of the efficacy of two methods of application or of two substances (milk and whey) the comparison was made with all plants as nearly as possible under the same environmental conditions. During the course of the experiments the greenhouse temperatures ranged from 51° to 91° F (mean 71° F).

The host material was Plush barley, free of seed-borne virus infection, which was treated with Captan 50 (50% Orthocide) against seedling blights and sown in 6-in. pots of autoclaved loam soil. A stock isolate of BSM, which had passed through several transfers on Plush barley seedlings, was used as inoculum. The whey or milk was applied either mixed with the inoculum previous to inoculation or as a fine spray to the exposed plant surfaces previous to inoculation. The spray was applied at the rate of 3.5 ml per 6-in. pot of

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Contribution No. 33 from the Canada Department of Agriculture Research Station, Winnipeg, Manitoba.
[2]Plant Pathology Laboratory.

seedlings by means of a de Vilbiss medicinal atomizer attached to a source of air at 15 p.s.i.

Inoculations were made by drawing sponge rubber pads moistened with inoculum and dusted with carborundum powder (600 mesh) over the primary leaf of plants whose secondary leaves were exposed not more than one inch. Adapted tongue-seizing forceps (8, fig. 3) were used to hold the pads. All necessary precautions were taken between the inoculations to avoid the cross transfer of inoculum or inhibitor. The forceps were immersed in a boiling water bath for 5 minutes, the hands were soaped and washed in water, and separate portions of inoculum were used for each treatment.

In the observations on infection, leaves of only one stage were compared at any given time, e.g. secondary leaf, tertiary leaf, etc. The following classes of systemic infection types, as indicated by the signs of infection on uninoculated leaves of inoculated plants, were used:

0 = no signs of infection; 1 = dashes or dots of chlorosis or necrosis; 2 = a mosaic of chlorotic dots and/or streaks, of chlorosis or necrosis, but with more than 50% of the leaf surface remaining green; and 3 = severe mosaic and/or chlorotic or necrotic streaks.

In the above classes the necrosis referred to is preceded by chlorosis.

In two preliminary experiments (I and II) the efficacy of milk as an inhibitor of BSM when mixed with the inoculum was compared with its efficacy when sprayed on the host previous to inoculation. In a third experiment, the efficacy of whey at four different concentrations applied as a spray was compared with milk at the same four concentrations. In a fourth experiment the retention of activity by whey during periods of storage in a cold room at about 0° C was tested.

The milk used in these experiments was an homogenized and pasteurized (155° F for 30 minutes) product sold in Winnipeg, Man., for household use. The whey was a by-product of cheddar-cheese production supplied fresh by the Dairy Science Department, University of Manitoba.

Freshly harvested Plush barley plants infected with stripe mosaic were used in all instances as a source of inoculum and the inoculum was prepared in essentially the same way in the different experiments. In experiment I, the entire pulped material was diluted to half strength (0.5) with either milk (inoculum designated IM) or water (IW). In experiment II, the infected plant material was diluted to 0.5 strength with distilled water, passed through filter paper, and divided into two portions. One portion was diluted to 0.25 strength with milk ($IM_2$) and the other to 0.25 with water ($IW_2$). In experiments III and IV the pulped material was first diluted with water to 0.5 strength then passed through filter paper and further diluted to 0.25 with water ($IW_3$ and $IW_4$). Experiment IV (Table III) was done to test the efficacy of stored whey whether unprocessed or processed to remove microorganisms. The Seitz filtrate and the overflow from a Sharple's centrifuge operated at 50,000 r.p.m. were dispensed into sterile medicine bottles with screw tops. All lots were stored at about 0° C.

In an exploratory field test, observations were made on the effect of skim milk and whey on the natural spread of infection from infected plants. The test plants were grown from uninfected seed in rows contiguous with plants grown from infected seed. Each plot consisted of two 5-ft rows of healthy plants bordered with one row on each side in which more than 50% of the plants were systemically infected with BSM. The rows were sown 6 in. apart within the plot and the plots were 4 ft apart, as in previous experiments no transmission of infection had occurred between plots sown three or more feet apart. All plots were sprayed weekly according to the frequencies indicated (Table IV) and at the rate of 40 gal (Imperial) per acre. All treatments were done in quadruplicate. When the kernels were in the milk stage, the plants in the two central rows were pulled and examined for signs of BSM.

## Results and Discussion

The results of three experiments are given in Tables I and II and illustrated in Fig. 1. It is evident that in these experiments milk had much the same effect in reducing the infection of BSM as it did in the experiments with TMV reported by others (1, 2, 3, 4, 6, 9). Milk inhibited infection whether it was sprayed on the plant several hours in advance of inoculation or mixed with the infective juice 10 minutes or 1 hour before inoculation. It was evident that whey was capable of causing about the same order of inhibition as was milk. When whey was diluted with water to 25% of its original concentration, a strong inhibition of infection occurred and even at a 3% concentration some inhibition occurred.

TABLE I

Infection from contact transmission of BSM in barley seedlings following treatment with milk

| Treatment | Inoculum | Number of plants | | | | | Weighted mean infection type | Proportionate infection* |
|---|---|---|---|---|---|---|---|---|
| | | Total | Infection type (secondary leaf) 0 | 1 | 2 | 3 | | |
| Experiment I (6th day after inoculation) | | | | | | | | |
| Inoculated with infective juice diluted with whole milk to 0.5 v/v | IM | 13 | 5 | 7 | 1 | 0 | 0.79 | 43 |
| Sprayed with whole milk, inoculated 3 hr later | IW | 16 | 7 | 6 | 3 | 0 | 0.75 | 40 |
| Inoculated with infective juice diluted with distilled water to 0.5 v/v (control) | IW | 14 | 1 | 0 | 13 | 0 | 1.85 | 100 |
| Experiment II (7th day after inoculation) | | | | | | | | |
| Infective juice diluted with distilled water to 0.5 v/v and then with milk to 0.25. Inoculation 10 min after dilution | $IM_2$ | 31 | 29 | 0 | 1 | 1 | 0.16 | 6 |
| Infective juice diluted with distilled water to 0.25 v/v. Inoculation 10 min after dilution | $IW_2$ | 36 | 0 | 0 | 9 | 27 | 2.75 | 100 |
| Inoculation with $IM_2$ 1 hr after dilution | $IM_2$ | 34 | 30 | 0 | 4 | 0 | 0.24 | 9 |
| Inoculation with $IW_2$ 1 hr after dilution | $IW_2$ | 38 | 1 | 1 | 7 | 29 | 2.68 | 100 |
| Sprayed with whole milk, inoculation 5 hr later | $IW_2$ | 38 | 36 | 0 | 2 | 0 | 0.11 | 4 |
| Sprayed with distilled water, inoculation 5 hr later | $IW_2$ | 40 | 0 | 0 | 9 | 31 | 2.77 | 100 |
| Stroked with carborundum without inoculum | 0 | 38 | 38 | 0 | 0 | 0 | 0 | 0 |
| Undisturbed | 0 | 39 | 39 | 0 | 0 | 0 | 0 | 0 |

*Mean infection type as a percentage of that of the control.

TABLE II

Infection from contact transmission of stripe mosaic in barley seedlings following spray applications of whey and milk at different concentrations (inoculum $IW_3$)*

| Treatment | Number of plants | | | | | Weighted mean infection type | Proportionate infection |
|---|---|---|---|---|---|---|---|
| | Total | Infection type (secondary leaf) 0 | 1 | 2 | 3 | | |
| | Experiment III (9th day)† | | | | | | |
| Sprayed with whey | | | | | | | |
| Concentration 100% | 16 | 15 | 0 | 0 | 1 | 0.19 | 7 |
| Concentration 25% | 19 | 0 | 0 | 0 | 0 | 0 | 0 |
| Concentration 6% | 16 | 8 | 2 | 5 | 1 | 0.94 | 34 |
| Concentration 3% | 17 | 5 | 0 | 5 | 7 | 1.82 | 66 |
| Sprayed with whole milk | | | | | | | |
| Concentration 100% | 15 | 15 | 0 | 0 | 0 | 0 | 0 |
| Concentration 25% | 14 | 14 | 0 | 0 | 0 | 0 | 0 |
| Concentration 6% | 14 | 7 | 2 | 4 | 1 | 0.93 | 34 |
| Concentration 3% | 16 | 10 | 1 | 5 | 0 | 0.69 | 25 |
| Sprayed with water (control) | 16 | 0 | 0 | 4 | 12 | 2.75 | 100 |

*Inoculations 4.5 hours after spray application.
†In subsequent readings made up to three weeks after inoculation, the relation of degree of infection to treatment remained substantially the same.

As will be noted in Tables I and II, inhibition was sometimes incomplete following treatment with whey or milk. The one plant in experiment III that became infected following a spray with 100% whey was not anomalous in view of the incomplete control that occurred with 100% whole milk in experiments I and II. The reason for an occasional escape of inhibition by one or a few plants is not evident. That it was not due to the presence of coarse infective particles in the pulped infected plants used as inoculum, which might protect the virus, is indicated in the results of experiments II and III, in which "escapes" occurred even though the infective juice was passed through filter paper. It seems improbable also, with the large volume of spray applied, that escape could have been attributable to any of the exposed plant surfaces remaining unsprayed. Adherence to the surface appeared to be uniform and complete.

The data in Table IV indicate that under field conditions both milk and whey are effective in reducing the plant-to-plant contact transmission of BSM. In the field experiment, a significant reduction in infection occurred following a single application of 100% whey, three applications of 20% whey, or four applications of 20% skim milk. The probability values for the single applications of 100% skim milk and 20% whey were respectively $P = 0.051$ and $P = 0.10$. The results were conclusive with respect to the inhibition of natural spread of stripe mosaic and will aid in designing further experiments with whey. They also demonstrate that 33% of uninfected barley plants

FIG. 1. Plush barley plants 14 days after inoculation with BSMV: (1) sprayed with a 25% solution of whey 4.5 hours before inoculation, and (2) sprayed with water 4.5 hours before inoculation.

PLATE I

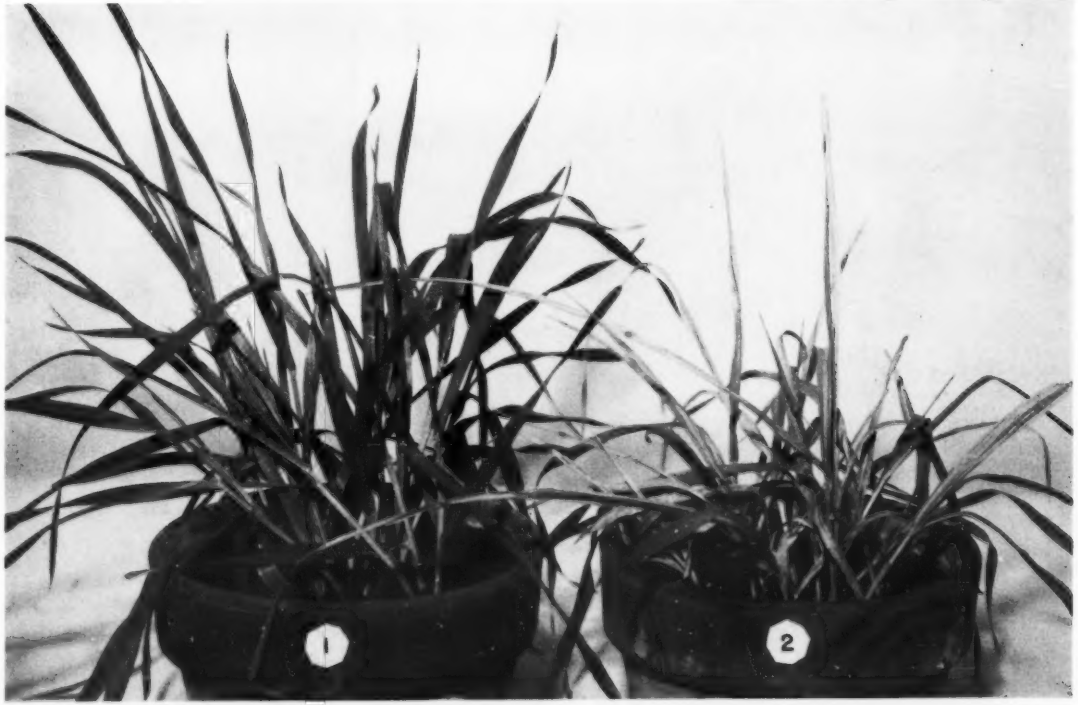


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TABLE III

Infection from contact transmission of BSM in barley seedlings following spraying with whey stored at about 0° C for periods of up to 24 days (inoculum $IW_4$)

| Date whey collected and/or processed | Method of processing | Period of storage, days | Weighted mean infection type (secondary leaves) | Proportionate infection |
|---|---|---|---|---|
| | Experiment IV (11th day) | | | |
| 8-VI-59 | None | 24 | 0* | 0 |
| 8-VI-59 | Seitz filtration | 24 | 0 | 0 |
| 16-VI-59 | None | 16 | 0 | 0 |
| 16-VI-59 | Sharple's supernatant (overflow, 50,000 r.p.m.) | 16 | 0 | 0 |
| Distilled water | — | — | 2.06 | 100 |

*One plant had infection in the tertiary leaf but not in the secondary leaf.

TABLE IV

Infection of BSM in Plush barley in the two central rows of the plot following natural spread from the two outside systemically infected rows

| Treatment (40 gal/acre) | | | Diseased plants, % |
|---|---|---|---|
| Material | Concentration, % | Number of applications | |
| Whey | 100 | 1 | 15** |
| Whey | 20 | 1 | 28 |
| Whey | 20 | 3 | 18** |
| Skim milk | 100 | 1 | 23 |
| Skim milk | 20 | 4 | 18** |
| Water | — | 4 | 33 |

**Significant at the 1% level.

may become infected from the natural spread from adjacent rows during a single season.

The fact that whey was effective against BSM probably indicates its general usefulness against any plant virus disease that may be inhibited by milk. Johnson (12) found that whey was capable of inhibiting local lesion development by TMV in *Nicotiana tabacum* × *N. glutinosum* and it seems probable that whey would be as effective as milk against TMV in tobacco.

## Acknowledgments

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# CHROMOSOME NUMBERS AND TAXONOMIC NOTES ON NORTHERN GRASSES

## II. TRIBE FESTUCEAE[1]

WRAY M. BOWDEN


## Abstract

Chromosome numbers and voucher specimens are recorded for some collections of the tribe FESTUCEAE Dumort., mainly collected in Canada. The somatic chromosome numbers of the following are recorded: (1*a*) *Arctagrostis arundinacea* (Trin.) Beal var. *arundinacea*, $2n = 28$, $2n = 29$, and $2n = 30$; (1*b*) *Arctagrostis arundinacea* (Trin.) Beal var. **crassispica** Bowden, $2n = 56$; (1*c*) *Arctagrostis latifolia* (R.Br.) Griseb., $2n = 56$. (2) *Beckmannia syzigachne* (Steud.) Fern., $2n = 14$. (3) *Brachyelytrum erectum* (Schreb.) Beauv., two vars., $2n = 22$. (4*a*) *Bromus ciliatus* L., $2n = 14$; (4*b*) *Bromus inermis* Leyss., two subspecies and two vars., $2n = 56$; (4*c*) *Bromus kalmii* A. Gray, $2n = 14$; (4*d*) *Bromus porteri* (Coult.) Nash, $2n = 14$; (4*e*) *Bromus tectorum* L., $2n = 14$. (5) *Catabrosa aquatica* (L.) Beauv. var. *laurentiana* Fern., $2n = 20$. (6) *Dactylis glomerata* L., $2n = 28$. (7) *Distichlis stricta* (Torr.) Rydb., $2n = 40$. (8*a*) *Dupontia fisheri* R.Br. subsp. *fisheri*, $2n = 132$; (8*b*) *Dupontia fisheri* R.Br. subsp. *psilosantha* (Rupr.) Hultén, $2n = 44$. (9) *Eremopoa persica* (Trin.) Roshev., $2n = 28$. (10*a*) *Festuca altaica* Trin., $2n = 28$; (10*b*) *Festuca baffinensis* Polunin, $2n = 28$; (10*c*) *Festuca brachyphylla* Schultes, $2n = 42$ and one collection, $2n = 44$; (10*d*) *Festuca elatior* L., $2n = 14$; (10*e*) *Festuca obtusa* Bieler, $2n = 42$; (10*f*) *Festuca prolifera* (Piper) Fern. var. *lasiolepis* Fern., $2n = 50$; (10*g*) *Festuca rubra* L., $2n = 42$; (10*h*) *Festuca saximontana* Rydb., $2n = 42$; (10*i*) *Festuca scabrella* Torr. ex Hook., two vars., $2n = 56$ and $2n = 28$. (11*a*) *Glyceria borealis* (Nash) Batchelder, $2n = 20$; (11*b*) *Glyceria canadensis* (Michx.) Trin., $2n = 60$; (11*c*) *Glyceria grandis* S. Wats., $2n = 20$; (11*d*) *Glyceria melicaria* (Michx.) Hubb., $2n = 40$; (11*e*) *Glyceria pulchella* (Nash) K. Schum., $2n = 20$; (11*f*) *Glyceria striata* (Lam.) Hitchc., $2n = 20$; (11*g*) *Glyceria* × **gatineauensis** Bowden (*G. melicaria* × *G. striata*), $2n = 30$; (11*h*) *Glyceria* × **ottawensis** Bowden (*G. canadensis* × *G. striata*), three nothomorphs, $2n = 42$, $2n = 46$, and $2n = 48$. (12) *Lolium rigidum* Gaud., $2n = 14$. (13*a*) *Phippsia algida* (Sol.) R.Br., $2n = 28$; (13*b*) *Phippsia concinna* (Th. Fries) Lindeb., $2n = 28$. (14) *Pleuropogon sabinei* R.Br., $2n = 42$. (15) *Schizachne purpurascens* (Torr.) Swallen, $2n = 20$. (16) *Torreyochloa fernaldii* (Hitchc.) Church, $2n = 14$.


## Introduction

In the first paper of this series on northern grasses, Bowden (1) reported the chromosome numbers of some collections of the tribe TRITICEAE Dumort. In the present paper, the second of the series, chromosome numbers and voucher specimens of 16 genera of the tribe FESTUCEAE Dumort. are recorded. The materials and methods have already been described by Bowden (1).

Pilger (21) included all of these 16 genera in the tribe FESTUCEAE, with the exception of *Torreyochloa* which was not listed. Darlington and Wylie (9) included 11 of these 16 genera in the tribe FESTUCEAE. They did not list the genus *Eremopoa*; and they included the genera *Arctagrostis*, *Phippsia*, *Beckmannia*, and *Brachyelytrum* in three other tribes. Pilger (21) rightly included *Phippsia* in the tribe FESTUCEAE. For the purposes of the present paper, I am including these 16 genera in the tribe FESTUCEAE Dumort. in the sense of

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Pilger (21), although it remains for future publications to place these genera in their appropriate tribes. Since a large number of collections of *Poa* and *Puccinellia* have been studied during this investigation, these two genera of the tribe FESTUCEAE will be considered separately in a later paper of this series.

## Results

The 16 genera and the species in each genus are arranged alphabetically so that the data can be easily consulted. For each voucher specimen, data are given on the province, district or state, the cytogenetic accession number (= Cyt. No.), the geographical locality where the material was originally collected, the collector's name and specimen number, and the somatic ($2n$) chromosome number. In the citation of chromosome numbers, the specimens sent in from the field as live plants are designated as "clones" and the plants grown to maturity from seed are designated as "plants". All of the voucher specimens are deposited in the Phanerogamic Herbarium of the Plant Research Institute (DAO).

(1*a*) *Arctagrostis arundinacea* (Trin.) Beal var. *arundinacea*

Five collections that appear to be the typical variety were tetraploid, $2n = 28$. One plant had one extra chromosome per cell and two plants had two extra chromosomes per cell. Britton and Rydberg (3) examined topotype specimens of *A. arundinacea* from Kotzebue's Sound, Alaska (NY), and I also examined two topotypes from the New York Botanical Garden Herbarium (NY; photographs, DAO). Plate 55 (*Vilfa arundinacea*) of Trinius (31), which is an iconotype, also matches the five specimens cited below fairly well.

Voucher specimens: Alaska: Cyt. No. 1368, Snow River Delta, south end of Kenai Lake, Kenai Peninsula, *J. A. Calder 6574*; $2n = 28$ det. on one plant and $2n = 30$ det. on two plants. Mackenzie District: Cyt. No. 1628, Norman Wells, *W. J. Cody & R. L. Gutteridge 7495*; $2n = 28$ det. on one clone. Cyt. No. 1692, Aklavik, Mackenzie River Delta, *W. J. Cody & R. L. Gutteridge 7927*; $2n = 28$ det. on one clone. Cyt. No. 1742, same locality, *W. J. Cody & R. L. Gutteridge 7905*; $2n = 28$ det. on one plant. Cyt. No. 1698, same locality, *W. J. Cody & R. L. Gutteridge 7928*; $2n = 29$ det. on one clone.

I have also examined the type specimens (NY; photographs, DAO) of *Arctagrostis angustifolia* Nash, *A. macrophylla* Nash, and *A. poaeoides* Nash, published in Britton and Rydberg (3), and they appear to me to be variations within var. *arundinacea*.

The general distribution of *A. arundinacea* has been given in Komarov's flora (17). Raup (23) has plotted the distribution of this species in Alaska and adjacent areas in Canada (his plate XVI).

(1*b*) *Arctagrostis arundinacea* (Trin.) Beal var. **crassispica** var. nov.

A typo differt spicis crassioribus, et culmis crassioribus, ad summas 2.0–3.0 mm.

The spikes and culms of specimens of var. *crassispica* are much coarser than those of var. *arundinacea*. The culms of var. *crassispica* are 2.0–3.0 mm in

diameter measured just below the bases of the spikes while the culms of var. *arundinacea* are (0.8)–1.5–(2.0) mm in diameter below the spikes. Two collections of var. *crassispica* were octoploid, $2n = 56$.

Voucher specimens of var. *crassispica*: Mackenzie District: Cyt. No. 1741, Norman Wells, large stand in moist ground by buildings, *W. J. Cody & R. L. Gutteridge 7808*, holotype and one isotype; $2n = 56$ det. on one plant. Cyt. No. 1252, Lower Hay River, *W. H. Lewis 6*, paratype; $2n = 56$, det. on one clone.

There is another specimen in the Phanerogamic Herbarium (DAO) which appears to be var. *crassispica* although the chromosome number has not been determined. The specimen is: Yukon: Coffee Creek, *M. O. Malte s.n., August 6, 1916*; paratype.

(1*c*) *Arctagrostis latifolia* (R.Br.) Griseb.

Five collections were octoploid, $2n = 56$.

Voucher specimens: Franklin District: Cyt. No. 579, Baffin Island, Frobisher Bay, *H. A. Senn s.n., July, 1948*; $2n = 56$ det. on one clone. Cyt. No. 616, same locality, *J. A. Calder s.n., August, 1948*; $2n = 56$ det. on one clone. Keewatin District: Cyt. No. 678, Southampton Island, Coral Harbor, *W. J. Cody 1998*; $2n = 56$ det. on one plant. Cyt. No. 1529 and 1530, same locality, *D. K. Brown s.n., September, 1952*; $2n = 56$ det. on two clones.

Flovik (10) reported $2n = 62$ for material of this species from Spitzbergen, and Holmen (12) observed $2n = 56$ in five collections from Greenland. Porsild (22) has mapped the distribution of *Arctagrostis latifolia* in northern North America (his map No. 17) and the complete Old and New World Arctic distribution has been summarized in Komarov's flora (17).

(2) *Beckmannia syzigachne* (Steud.) Fern.

Plants of six collections were diploid, $2n = 14$.

Voucher specimens: Manitoba: Cyt. No. 671, Fort Churchill area, *J. M. Gillett s.n., August, 1948*; $2n = 14$ det. on three clones. Mackenzie District: Cyt. No. 892, Yellowknife, *W. J. Cody & J. B. McCanse 3023*; $2n = 14$ det. on one clone. Cyt. No. 1079, Salt River, *W. J. Cody & C. C. Loan 4597*; $2n = 14$ det. on one clone. Cyt. No. 1250, Lower Hay River, *W. H. Lewis 4*; $2n = 14$ det. on one clone. Cyt. No. 1697, Aklavik, *W. J. Cody & R. L. Gutteridge 7933*; $2n = 14$ det. on one clone. Cyt. No. 1738, Aklavik, *W. J. Cody & R. L. Gutteridge 7913*; $2n = 14$ det. on one plant.

Nielsen and Humphrey (20) and Tateoka (27) reported $2n = 14$ for *B. syzigachne*.

(3) *Brachyelytrum erectum* (Schreb.) Beauv.

Four collections of this species were diploid, $2n = 22$. Three collections were var. *erectum*, and one (Cyt. No. 1505) was var. *septentrionale* Babel.

Voucher specimens: Ontario: Cyt. No. 1505, Frontenac Co.: near McLean, *W. G. Dore & K. Mills 13793*; $2n = 22$ det. on one clone. Cyt. No. 1513, Frontenac Co.: Sharbot Lake area, *J. M. Gillett & W. G. Dore 6710*; $2n = 22$ det. on one clone. Cyt. No. 1759, Carleton Co.: 2 miles SW. of Manotick

Station, *W. M. Bowden & W. G. Dore 82-53*; $2n = 22$ det. on one clone. Wisconsin: Cyt. No. 1750, Green Co.: Attica Woods, *W. G. Dore 14767*; $2n = 22$ det. on one plant.

Brown (4) reported $2n = 22$ for *B. erectum*.

(4*a*) *Bromus ciliatus* L.

One collection was diploid, $2n = 14$.

Voucher specimen: Ontario: Cyt. No. 1853, Carleton Co.: between Harwood Plains and Shirley Bay, *W. M. Bowden 122-55*; $2n = 14$ det. on one clone.

Wagnon (32) reported $2n = 14$ for numerous collections of this species.

(4*b*) *Bromus inermis* Leyss.

Six collections were octoploid, $2n = 56$. Three collections (Cyt. No. 1052, 1068, and 1251) were subsp. *inermis*; two collections (Cyt. No. 1121 and 1621) were subsp. *pumpellianus* (Scribn.) Wagnon var. *pumpellianus* (= var. *purpurascens* (Hook.) Wagnon); and one (Cyt. No. 1120) was subsp. *pumpellianus* (Scribn.) Wagnon var. *arcticus* (Shear) Wagnon.

Voucher specimens: Labrador: Cyt. No. 1068, Goose Bay, *J. M. Gillett s.n., July, 1950*; $2n = 56$ det. on one clone. Mackenzie District: Cyt. No. 1052, Fort Smith, *W. J. Cody & C. C. Loan 3938*; $2n = 56$ det. on one clone. Cyt. No. 1251, Lower Hay River, *W. H. Lewis 5*; $2n = 56$ det. on one clone. Cyt. No. 1120, Fort Smith, *C. C. Loan 115*; $2n = 56$ det. on two plants. Cyt. No. 1621, Norman Wells, *W. J. Cody & R. L. Gutteridge 7496*; $2n = 56$ det. on one clone. Alberta: Cyt. No. 1121, Halfway, 7 miles south of Fort Smith, *C. C. Loan 81*; $2n = 56$ det. on two plants.

Wagnon (32) reported $2n = 56$ or ca. 56 for all of his collections of this species, including the subspecies and varieties listed above.

(4*c*) *Bromus kalmii* A. Gray

One collection was diploid, $2n = 14$.

Voucher specimen: Ontario: Cyt. No. 1459, Lambton Co.: about 4 miles NE. of Port Frank, *J. H. Soper & J. K. Shields 5038*; $2n = 14$ det. on one plant.

Wagnon (32) observed $2n = 14$ in collections of *B. kalmii* from Michigan and Indiana.

(4*d*) *Bromus porteri* (Coult.) Nash

One collection was diploid, $2n = 14$.

Voucher specimen: Manitoba: Cyt. No. 376, Shilo Camp, *R. O. Bibbey & C. Frankton 6*; $2n = 14$ det. on one clone.

Wagnon (32) reported $2n = 14$ for numerous collections of *B. porteri*. Hitchcock (11) listed this species under *Bromus anomalus* Rupr. ex Fourn. but Wagnon (32) considered that the latter species occurs from western Texas and Baja California to Mexico.

(4*e*) *Bromus tectorum* L.

One collection was diploid, $2n = 14$.

Voucher specimen: Alaska: Cyt. No. 1369, about 3 miles NE. of Kasilof, Kenai Peninsula, *J. A. Calder 6698*; $2n = 14$ det. on one plant.

The chromosome number $2n = 14$ was reported for *B. tectorum* by de Cugnac and Simonet (8), Knowles (16), and Tateoka (29).

(5) *Catabrosa aquatica* (L.) Beauv. var. *laurentiana* Fern.

One collection was diploid, $2n = 20$.

Voucher specimen: Ontario: Cyt. No. 1571, Berge glaiseuse et humide de la rivière, Albany, *E. Lepage 30, 208*; $2n = 20$ det. on one plant.

Löve and Löve (18) summarized the data of four authors and all reported $2n = 20$ for *C. aquatica*.

(6) *Dactylis glomerata* L.

Four collections from Gander, Newfoundland, were tetraploid, $2n = 28$.

Voucher specimens: Newfoundland: Gander: Cyt. No. 810, 816, 844, and 853, *I. J. Bassett s.n., July, 1949*; $2n = 28$ det. on four clones.

Löve and Löve (18) listed numerous authors who have reported $2n = 28$ for this species.

(7) *Distichlis stricta* (Torr.) Rydb.

One collection was tetraploid, $2n = 40$.

Voucher specimen: Mackenzie District: Cyt. No. 1072, Salt Plain west of Ft. Smith, *W. J. Cody & C. C. Loan 4570*; $2n = 40$ det. on one clone.

Stebbins and Love (26) recorded $2n = 40$ for this species.

(8*a*) *Dupontia fisheri* R.Br. subsp. *fisheri*

One collection had the somatic chromosome number $2n = 132$.

Voucher specimen: Keewatin District: Cyt. No. 707, Southampton Island, Coral Harbor, *W. J. Cody 1929*; $2n = 132$ det. on one plant.

Several polyploids with different chromosome numbers have been reported for this species; see discussion under the next subspecies. Map No. 43 of Porsild (22) shows the geographical range of subsp. *fisheri*.

(8*b*) *Dupontia fisheri* R.Br. subsp. *psilosantha* (Rupr.) Hultén

Three collections had the somatic chromosome number $2n = 44$.

Voucher specimens: Franklin District: Cyt. No. 575, Baffin Island, Frobisher Bay, *H. A. Senn s.n., July, 1948*; $2n = 44$ det. on one clone. Cyt. No. 614 and 615, Baffin Island, Frobisher Bay, *J. A. Calder s.n., August, 1948*; $2n = 44$ det. on two clones.

Flovik (10) reported $2n = 88 + \text{ff}$ for *D. fisheri* and $2n = 44 + \text{ff}$ for *D. fisheri* R. Br. var. *psilosantha* (Rupr.) Schol. from Spitzbergen. Sokolovskaya and Strelkova (25) listed $2n = 42$ for material of *D. fisheri* from Kolguev Island and $2n = 88$ for material from Spitzbergen. Thus, with the new polyploid listed under (8*a*), there are at least three polyploids in the *Dupontia fisheri* complex. Much more material is needed to assess this problem properly. Porsild (22) has mapped the geographical range of subsp. *psilosantha* in his map No. 44.

(9) *Eremopoa persica* (Trin.) Roshev.

One collection was tetraploid, $2n = 28$.

Voucher specimen: Manitoba: Cyt. No. 1896, Brandon, west end of C.N.R. yard about McTavish and Eight Street, *G. A. Stevenson 945*; $2n = 28$ det. on four plants (specimens preserved); a newly detected introduction.

The genus *Eremopoa* has been monographed in Komarov's (17) Flora U.S.S.R.

(10*a*) *Festuca altaica* Trin.

Three collections were tetraploid, $2n = 28$.

Voucher specimens: Alaska: Cyt. No. 1399, Big Delta Camp area, *W. J. Cody 6256*; $2n = 28$ det. on one plant. Cyt. No. 1394, Big Delta Camp area, *W. J. Cody & T. J. M. Wesbter 6155*; $2n = 28$ det. on one plant. Mackenzie District: Cyt. No. 1757, Mackenzie Mts., Mile 44E, Canol Hy., *W. J. Cody & R. L. Gutteridge 7717*; $2n = 28$ det. on one plant.

Sokolovskaya and Strelkova (24) reported $2n = 28$ for *F. altaica* Trin. from the Altai region. In Plate XV of Raup (23), the geographical distribution of this species in northwestern North America is shown.

(10*b*) *Festuca baffinensis* Polunin

Six collections of this species were tetraploid, $2n = 28$.

Voucher specimens: Franklin District: Cyt. No. 1452, Spence Bay, settlement, *J. G. Chillcott 79*; $2n = 28$ det. on one plant. Cyt. No. 1682, Axel Heiberg Island, *A. E. Porsild s.n.*; $2n = 28$ det. on one clone. Keewatin District: Cyt. No. 679, 697, 704, and 705, four different localities, Southampton Island, Coral Harbor, *W. J. Cody 2014, 1705, 1952, and 1971*; $2n = 28$ det. on four plants.

Holmen (12) reported $2n = 28$ for a Greenland collection that he thought might be *F. baffinensis*. A duplicate specimen of Holmen's collection (No. 8077 from Brønlund Fjord) has been deposited in our herbarium (DAO) and there is no doubt that the specimen is *F. baffinensis*. The geographical range of this species has been mapped by Porsild (22) in his map No. 54.

(10*c*) *Festuca brachyphylla* Schultes

Ten collections of this species were hexaploid, $2n = 42$, and one collection (Cyt. No. 694) was hexaploid with two extra chromosomes ($2n = 44$).

Voucher specimens: Alaska: Cyt. No. 1373, Victor Creek, Lake Kenai, Kenai Peninsula, *J. A. Calder 5810*; $2n = 42$ det. on one plant. Cyt. No. 1374, Falls Creek Mine, near Kenai Lake, Kenai Peninsula, *J. A. Calder 6179*; $2n = 42$ det. on one plant. Keewatin District: Cyt. No. 701, Southampton Island, Coral Harbor, *W. J. Cody 1898*; $2n = 42$ det. on one plant. Cyt. No. 1103 and 1104, Chesterfield Inlet, $\frac{1}{4}$ mile SW. of settlement, *D. B. O. Savile & C. T. Watts 1549 & 1550*; Cyt. No. 1104 is f. *flavida* Polunin; $2n = 42$ det. on two plants. Franklin District: Cyt. No. 598 and 600, Baffin Island, Frobisher Bay, *J. A. Calder s.n., August, 1948*; $2n = 42$ det. on two clones. Quebec: Cyt. No. 694 and 695, Fort Chimo area, *J. A. Calder 2247 & 2393*; $2n = 42$

det. on one plant and $2n = 44$ det. on one plant (Cyt. No. 694). Cyt. No. 1101, Lac Payne, lobe de l'est, *J. Rousseau 883*; $2n = 42$ det. on one plant. Cyt. No. 1002, Baie Kayak, dans l'estuaire de la baie Payne, *J. Rousseau 1528*; $2n = 42$ det. on one plant.

Holmen (12) reported $2n = 42$ for *F. brachyphylla* from Greenland. Map No. 53 of Porsild (22) and the map (Plate XV) of Raup (23) show the geographical range of this species.

(10*d*) *Festuca elatior* L.

Three collections were diploid, $2n = 14$.

Voucher specimens: Newfoundland: Cyt. No. 815, Gander, *I. J. Bassett s.n., July, 1949*; $2n = 14$ det. on one clone. Nova Scotia: Cyt. No. 1483, Inverness Co.: Glendyer, *E. C. Smith et al. 4796*; $2n = 14$ det. on one plant. Ontario: Cyt. No. 1575, Napanee, *W. G. Dore 13836*; $2n = 14$ det. on one plant.

Myers and Hill (19) and Tateoka (28) reported $2n = 14$ for *F. elatior*.

(10*e*) *Festuca obtusa* Bieler

Five collections were hexaploid, $2n = 42$.

Voucher specimens: Quebec: Argenteuil Co.: Cyt. No. 1849, 1 mile north of Cushing, *L. Jenkins 5707*; $2n = 42$ det. on one clone. Ontario: Cyt. No. 1845, Carleton Co.: between Harwood Plains and Shirley Bay, *W. M. Bowden & L. Jenkins 114-55*; $2n = 42$ det. on one clone. Cyt. No. 1847, Russell Co.: near Navan, *W. G. Dore & L. Jenkins 15867*; $2n = 42$ det. on one clone. Cyt. No. 1843, Leeds Co.: along Jones Creek near St. Lawrence River, 8 miles SW. of Brockville, *W. G. Dore & J. M. Gillett 15842*; $2n = 42$ det. on one clone. Cyt. No. 1865, Norfolk Co.: Turkey Point, near Municipal Park, *W. M. Bowden 140-55*; $2n = 42$ det. on one clone.

(10*f*) *Festuca prolifera* (Piper) Fern. var. *lasiolepis* Fern.

One collection had the somatic chromosome number $2n = 50$. Since this was an unusual number, several different fixations were made and slides were prepared several times but the count was always $2n = 50$.

Voucher specimen: Quebec: Cyt. No. 646, Fort Chimo area, *J. A. Calder s.n., August, 1948*; $2n = 50$ det. on one clone.

(10g) *Festuca rubra* L.

Six collections were hexaploid, $2n = 42$.

Voucher specimens: Newfoundland: Cyt. No. 1416, Head of Harbour, St. Anthony, *D. B. O. Savile & J. Vaillancourt 2887*; $2n = 42$ det. on one plant. Cyt. No. 832, 837, and 845, Gander, *I. J. Bassett s.n., July, 1949*; $2n = 42$ det. on three clones. Prince Edward Island: Cyt. No. 1499, Queens Co.: Bunbury near Charlottetown, *D. Erskine & W. G. Dore 1157*; $2n = 42$ det. on one clone. Mackenzie District: Cyt. No. 1868, Fort Simpson, edge of field, Experimental Farm, *W. J. Cody & J. M. Matte 8791*; $2n = 42$ det. on one clone.

Löve and Löve (18) listed numerous authors who reported $2n = 42$ for this species.

(10*h*) *Festuca saximontana* Rydb.

Twelve collections were hexaploid, $2n = 42$.

Voucher specimens: Alaska: Cyt. No. 1398, Mile 15, Paxton-Cantwell Highway, *T. J. M. Webster 57*; $2n = 42$ det. on one plant. Yukon: Cyt. No. 761, Whitehorse, *J. M. Gillett s.n., June, 1949*; $2n = 42$ det. on one clone. Cyt. No. 987, about 3 miles northeast of Mayo, *J. A. Calder, J. M. Gillett et al. 4099*; $2n = 42$ det. on one plant. Saskatchewan: Cyt. No. 1823, 10 milles à l'ouest de Frenchman Butte, *B. Boivin & D. Dunbar 10498*; $2n = 42$ det. on one clone. Mackenzie District: Yellowknife: Cyt. No. 887, *W. J. Cody & J. B. McCanse 3030*; $2n = 42$ det. on one clone. Cyt. No. 889, same locality, *W. J. Cody & J. B. McCanse 3028*; $2n = 42$ det. on one clone. Cyt. No. 891, same place, *W. J. Cody & J. B. McCanse 3033*; $2n = 42$ det. on one clone. Cyt. No. 913, Yellowknife, Jackfish Lake, *W. J. Cody & J. B. McCanse 3205*; $2n = 42$ det. on one plant. Cyt. No. 914, Yellowknife, airstrip, *W. J. Cody & J. B. McCanse 3237*; $2n = 42$ det. on one plant. Cyt. No. 916, Yellowknife, airstrip, *W. J. Cody & J. B. McCanse 3239*; $2n = 42$ det. on one plant. Cyt. No. 984, Yellowknife, Joliffe Island, *W. J. Cody & J. B. McCanse 3093*; $2n = 42$ det. on one plant. Cyt. No. 983, Indin Lake, *W. J. Cody & J. B. McCanse 3462*; $2n = 42$ det. on one plant.

(10*i*) *Festuca scabrella* Torr. ex Hook.

Three collections of *F. scabrella* var. *major* Vasey were octoploid, $2n = 56$, and one collection (var. ?) was tetraploid, $2n = 28$.

Voucher specimens of the octoploids (var. *major*): Alberta: Cyt. No. 1443, 6 miles north of entrance to Waterton Lakes National Park, *W. G. Dore 12141*; $2n = 56$ det. on one plant. Cyt. No. 1444, 5 miles east of Pincher Creek, *W. G. Dore 11637*; $2n = 56$ det. on one plant. Cyt. No. 1561, grassy slope at the "Hoodoos", Banff, *L. Jenkins 1395*; $2n = 56$ det. on one plant. All three specimens have reddish areas near the culm bases.

Voucher specimens of the tetraploid (var. ?): Cyt. No. 1560, Quebec: Ungava Bay Watershed: Berge sableuse de la riv. aux Mélèzes, 2 milles en haut de la fourche, Lat. 58° 41′, *Dutilly, Lepage, & Duman 28, 171*; $2n = 28$ det. on three plants.

Johnston (15) mapped the distribution of *F. scabrella*. He showed that this species occupies a large area in western North America and that it has disjunct stations in eastern North America. A much larger sampling of cytologically determined specimens needs to be on hand before this species can be understood. There is no doubt that there is morphological variation in the specimens of this species in our herbarium as well as those mapped in Fig. 1 of Johnston (15). The cytological evidence confirms this opinion since the three Alberta collections were octoploid while the collection from northern Quebec was tetraploid. Hultén (13) considered that *F. scabrella* is closely related to *F. altaica*.

(11*a*) *Glyceria borealis* (Nash) Batchelder

One collection of this species was diploid, $2n = 20$.

Voucher specimen: Ontario: Carleton Co.: Cyt. No. 1851, between Harwood Plains and Shirley Bay, *W. M. Bowden 120-55*; $2n = 20$ det. on one clone.

Church (5) reported $2n = 20$ for this species.

(11*b*) *Glyceria canadensis* (Michx.) Trin.

Eight collections were hexaploid, $2n = 60$.

Voucher specimens: Nova Scotia: Cyt. No. 1892, Armdale, suburb of Halifax, *D. Erskine s.n., August 7, 1955*; $2n$ = ca. 60 det. on one clone. Quebec: Cyt. No. 1858 and 1859, Gatineau Park road, 3.3 miles north of Eardley, *W. M. Bowden & W. G. Dore 126-55 and 127-55*; $2n = 60$ det. on two clones. Cyt. No. 1890, 90 yards east of Gatineau Park road, 3.3 miles north of Eardley, *W. M. Bowden & L. Jenkins 171-55*; $2n$ = ca. 60 det. on one clone. Ontario: Cyt. No. 1831, Carleton Co.: Mer Bleue, at end of Borthwick road, 3 miles SE. of Blackburn Station, *W. G. Dore & W. M. Bowden 15354*; $2n$ = ca. 60 det. on one clone. Cyt. No. 1884, same locality, *W. M. Bowden & L. Jenkins 163-55*; $2n$ = ca. 60 det. on one clone. Cyt. No. 1886, same locality, *W. M. Bowden & L. Jenkins 165-55*; $2n$ = ca. 60 det. on one clone. Cyt. No. 1684, Algoma, Haviland Bay on Hy. 17 north of Sault Ste. Marie and near Pancake Bay, *W. M. Bowden 47-53*; $2n = 60$ det. on one clone.

Two panicles each of Cyt. No. 1858 and Cyt. No. 1884 (plot plants) were bagged and self-pollinated. Many seeds were observed in the mature panicles (attached to preserved specimens). The specimen of Cyt. No. 1892 from Nova Scotia has smaller spikelets and there are apparently no seeds present. This specimen seems to fit the taxon sometimes called *G. laxa* Scribn. or *G. canadensis* (Michx.) Trin. var. *laxa* (Scribn.) Hitchc. Some specimens (*laxa*) from Nova Scotia do have seeds present (DAO!).

Church (5) reported $2n = 60$ for both *G. canadensis* and *G. canadensis* var. *laxa*.

(11*c*) *Glyceria grandis* S. Wats.

Six collections of this species were diploid, $2n = 20$.

Voucher specimens: Quebec: Cyt. No. 1863, Gatineau Park road, 3.3 miles north of Eardley, moist roadside ditch, *W. M. Bowden & W. G. Dore 131-55*; $2n = 20$ det. on one clone. Cyt. No. 1891, same locality, growing in the shade at edge of woods, *W. M. Bowden & L. Jenkins 172-55*; $2n = 20$ det. on one clone. Ontario: Carleton Co.: Cyt. No. 1808, Mer Bleue, at end of Borthwick road, 3 miles SE. of Blackburn Station, *W. G. Dore & L. Jenkins 15204*; $2n = 20$ det. on one clone. Cyt. No. 1809, same locality, f. *pallescens* Fern., *W. G. Dore & L. Jenkins 15205*; $2n = 20$ det. on one clone. Saskatchewan: District of Weyburn: Cyt. No. 1476, 1 mille au nord de Minton, *B. Boivin 8662*; $2n = 20$ det. on one plant. Mackenzie District: Cyt. No. 925, Yellowknife, *W. J. Cody & J. B. McCanse 3557*; $2n = 20$ det. on one plant.

One panicle of Cyt. No. 1863 (plot plant) was bagged and self-pollinated. Many seeds were present in the mature panicle (attached to preserved specimen).

Church (5) reported $2n = 20$ for *G. grandis*.

(11*d*) *Glyceria melicaria* (Michx.) Hubb.

Two collections were tetraploid, $2n = 40$.

Voucher specimens: Quebec: Cyt. No. 1895, Lac Superieur, Terrebonne County, extensive stand in spring-fed roadside ditch, rare, seeds present, *W. G. Dore & J. M. Matte 16242*; $2n = 40$ det. on one clone. Cyt. No. 2547, Gatineau Park road, 3.5 miles north of Eardley, just north of rise (hill) in road, small immature clump, just inside edge of woods, east of road and just east of roadside ditch, *W. M. Bowden 1-59-3*; $2n = 40$ det. on roots fixed from living specimen.

Church (5) observed $2n = 40$ in this species.

(11*e*) *Glyceria pulchella* (Nash) K. Schum.

Three collections of this species were diploid, $2n = 20$.

Voucher specimens: Saskatchewan: Cyt. No. 1463 and 1568, Hoosier, *L. Jenkins 1078*; $2n = 20$ det. on two plants. Mackenzie District: Cyt. No. 1740, Norman Wells, *W. J. Cody & R. L. Gutteridge 7537*; $2n = 20$ det. on one plant. Cyt. No. 1876, Fort Simpson, *W. J. Cody & J. M. Matte 8774*; $2n = 20$ det. on one clone.

(11*f*) *Glyceria striata* (Lam.) Hitchc.

Four collections of this species were diploid, $2n = 20$.

Voucher specimens: Quebec: Cyt. No. 1862, Gatineau Park road, 3.3 miles north of Eardley, *W. M. Bowden & W. G. Dore 130-55*; $2n = 20$ det. on one clone. Ontario: Carleton Co.: Cyt. No. 1812, Mer Bleue, at end of Borthwick road, 3 miles SE. of Blackburn Station, *W. G. Dore & L. Jenkins 15208*; $2n = 20$ det. on one clone. Cyt. No. 1846, just east of Mud Pond between Constance Lake and Shirley Bay, *W. M. Bowden & L. Jenkins 115-55*; $2n = 20$ det. on four clones. Norfolk Co.: Charlotteville Twp.: Cyt. No. 1826, Lot 10, Concn. II, *W. M. Bowden 88-54*; $2n = 20$ det. on one clone.

Three panicles of Cyt. No. 1862 (plot plant) were bagged and self-pollinated. Many seeds were present in the mature panicles (attached to preserved specimen).

Church (5) reported $2n = 20$ for some collections of *G. striata*.

(11*g*) *Glyceria* × **gatineauensis** hybr. nov.

(*Glyceria melicaria* × *G. striata*)

Culmae numerosae, paniculae aliquantulum angustatae, ramibus longis (ad 12.0 cm long.), lemmatibus nervis rugosis, glumis millimetrum excedentibus.

This hybrid has numerous culms, somewhat contracted panicles with long branches (up to 12.0 cm long), lemmas with prominent nerves, and the glumes longer than 1 mm. In comparison, specimens of *Glyceria melicaria* have much contracted (narrow) panicles with short lateral branches (4.0 to 6.5 cm long), glumes longer than 1 mm, and faintly nerved lemmas; and specimens of *G. striata* have open panicles with long branches (6.0 to 10.0 cm long), glumes shorter than 1 mm, and prominently nerved lemmas.

Three collections of this hybrid were triploid, $2n = 30$, which is intermediate between the two putative parents (*G. melicaria*, $2n = 40$, and *G. striata*, $2n = 20$).

Voucher specimens: Quebec: Cyt. No. 1857, about 90 yards east of Gatineau Park road, 3.3 miles north of Eardley, dense stand on banks of drainage ditch, *W. M. Bowden & W. G. Dore 125-55*; $2n = 30$ det. on two clones from same patch; 1857 C1, holotype; 1857 C2, isotype. Cyt. No. 2545, Gatineau Park road, 3.5 miles north of Eardley, just north of rise (hill) in road, large clump, immature, roadside ditch, east side of road, *W. M. Bowden 1-59-1*; $2n = 30$ det. on roots fixed from living plant. Cyt. No. 2546, same area, immature, a different large clump, *W. M. Bowden 1-59-2*; $2n = 30$ det. on roots fixed from living specimen; 2545 and 2546, paratypes; also two hortotypes (more mature).

No seeds were present in the numerous spikelets of the original collection of 1857 C1 and 1857 C2 (June 29, 1955) and when the plants were examined at the same site on August 9, 1955, no seeds could be observed. One panicle of 1857 C1 (plot plant) was bagged and self-pollinated; no seeds could be seen in the mature panicle (attached to preserved specimen).

(11*hi*) *Glyceria* × **ottawensis** hybr. nov.
(*Glyceria canadensis* × *G. striata*)

Paniculae laxae, spiculis 3.5–4.0 mm long., lemmatibus nervis distinctis sed non rugosis, glumis millimetrum excedentibus.

The hybrid clump covered a large area (about 50 ft across). The specimens of *G.* × *ottawensis* usually have open panicles, spikelets 3.5–4.0 mm long, nerves of the lemmas evident but not prominent, and the glumes more than 1 mm long. In comparison, *Glyceria canadensis* has open, lax panicles, spikelets 5.0 to 6.0 mm long, nerves of the lemmas evident but not prominent, and the glumes more than 1 mm long; and specimens of *G. striata* have open panicles, spikelets 3.0 to 4.0 mm long, nerves of the lemmas prominent, and the glumes shorter than 1 mm.

Two collections from the same hybrid patch were tetraploid with two extra chromosomes ($2n = 42$). The hybrid is intermediate between the two putative parents (*G. canadensis*, $2n = 60$, and *G. striata*, $2n = 20$). Since two different hybrids (nothomorphs) with presumably the same species parentage are listed under (11*hii*), the $2n = 42$ chromosome hybrid is nm. *ottawensis*.

Voucher specimens of *G.* × *ottawensis* nm. *ottawensis*: Ontario: Cyt. No. 1810, Carleton Co.: Mer Bleue, at end of Borthwick road, 3 miles SE. of Blackburn Station, among alders in sodden soil along margin of bog, a single large patch, evidently a long established clone, very rare, *W. G. Dore & L. Jenkins 15206*; (holotype, isotypes, and two hortotypes); $2n = 42$ det. on one clone. Cyt. No. 1885, same patch, about 50 ft across, rhizomatous and spreading, sterile (no seed) small spikelets, *W. M. Bowden & L. Jenkins 164-55*; two sheets, topotypes; $2n =$ ca. 42 det. on one clone.

Two panicles of 1810 (plot plant) were bagged and self-pollinated. No seeds could be found in any of the panicles.

(11*hii*) *Glyceria* × *ottawensis* Bowden, two other nothomorphs

Three other collections of different hybrid plants which appear to have the same parentage as nm. *ottawensis* were examined. One collection was tetraploid with six extra chromosomes ($2n = 46$) and two collections were tetraploid with eight extra chromosomes ($2n = 48$). These three collections appear to be nothomorphs (hybrid forms) and I am not giving them separate nothomorph names since they can be readily designated by their different chromosome numbers.

Voucher specimens: Ontario: Cyt. No. 1887, Carleton Co.: Mer Bleue, at end of Borthwick road, 3 miles SE. of Blackburn Station, south of bog, dense stand along old fence, near and just north of end of road, half-way down the hillside, in very moist area, very dense stand and many flowering culms, sterile (no seed), *W. M. Bowden & L. Jenkins 166-55*; $2n = 46$ det. on one clone. Quebec: Cyt. No. 1888 and 1889, along ditch, 100 yards east of Gatineau Park road, 3.3 miles north of Eardley, no seeds present in spikelets, *W. M. Bowden & L. Jenkins 169-55 and 170-55*; $2n$ = ca. 48 in two clones. The 48-chromosome hybrid has stronger morphological characteristics of *G. canadensis* than the 42-chromosome hybrid (nm. *ottawensis*).

Two panicles of Cyt. No. 1887 (plot plant) were bagged and self-pollinated. No seeds could be found in any of the panicles.

The above (11*hi* and 11*hii*) three nothomorphs ($2n = 42$, $2n = 46$, and $2n = 48$) represent three different sterile hybrids. One of the parents was almost certainly *G. canadensis* ($2n = 60$) and the odd number of chromosomes in the hybrids probably came from this parent. The relatively high chromosome numbers of the hybrids and the morphology of the hybrid specimens are strong evidence that *G. canadensis* was probably one of the parents. The other parent is difficult to state finally without specimens of artifical interspecific hybrids for comparison, but it appears to have been *G. striata* ($2n = 20$).

Many more cytologically determined specimens of *Glyceria* species and interspecific hybrids are needed before these specimens can be adequately treated. When the plants are just beginning to bloom in June in the Ottawa area, I have noted that there are numerous new root tips that are satisfactory for cytological study. The best way is to fix root tips from each freshly dug field specimen and then preserve the numbered specimen from which the roots are taken. In that way, one can be certain that the chromosome number applies to the designated specimen. Artificial interspecific hybrids are needed to confirm the putative parentages listed above. The spikelets are small and delicate in these *Glyceria* species. It might be easier to make crosses if self-sterile plants of the parental species can be found, although it would be preferable also to have hybrids derived from normal parental specimens.

(12) *Lolium rigidum* Gaud.

One collection was diploid, $2n = 14$.

Voucher specimen: Saskatchewan: Maple Creek District: 7 à 10 milles au nord de Maple Creek, cultivated by J. B. Campbell, introduced from

Australia, *B. Boivin, J. B. Campbell, & J. M. Gillett 8928*; $2n = 14$ det. on one plant.

Jenkin and Thomas (14) and Tateoka (29) reported $2n = 14$ for this species.

(13*a*) *Phippsia algida* (Sol.) R.Br.

Plants of six collections were tetraploid, $2n = 28$.

Voucher specimens: Sweden: Cyt. No. 1634, Mt. Nuolja, Abisko, about 1100 m.s.m., *A. Nygren s.n., July, 1953*; $2n = 28$ det. on seedlings that germinated from soil. Colorado: Cyt. No. 1550, Clear Creek Co.: north slope of Mount Evans, *W. A. Weber s.n., Oct. 5, 1952*; $2n = 28$ det. on three clones. Franklin District: Cyt. No. 586, Baffin Island, Frobisher Bay, *H. A. Senn & J. A. Calder 3810*; $2n = 28$ det. on four plants. Cyt. No. 995, Cornwallis Island, Resolute Bay, *W. B. Schofield 623*; $2n = 28$ det. on one plant. Cyt. No. 1021, Cornwallis Island, Resolute Bay, *W. B. Schofield 431*; $2n = 28$ det. on one plant. Cyt. No. 1447, 2 miles west of Cape Belknap, northeast of Ellesmere Island, *P. F. Bruggemann 240*; $2n = 28$ det. on one plant.

Löve and Löve (18) listed five papers which reported $2n = 28$ for *P. algida* and they found the same number in Icelandic material. Map No. 16 of Porsild (22) shows the range of this species (including *P. concinna*) in North America. In Komarov's (17) Flora U.S.S.R., the general distribution of *P. algida* has been stated.

(13*b*) *Phippsia concinna* (Th. Fries) Lindeb.

Three collections of this species were tetraploid, $2n = 28$.

Voucher specimens: Franklin District: Cyt. No. 1537, Mould Bay, Prince Patrick Island, *P. F. Bruggemann 412*; $2n = 28$ det. on one plant. Cyt. No. 1538, same locality, *P. F. Bruggemann 400*; $2n = 28$ det. on one plant. Cyt. No. 1543, same locality, *P. F. Bruggemann 399*; $2n = 28$ det. on one plant.

Flovik (10) reported $2n = 29$ for material of *P. concinna* var. *algidiformis* (Smith) Holm.

*Phippsia concinna* is undoubtedly closely related to *P. algida*, the preceding species. Since several contemporary botanists recognize both species, I am reporting them separately in this paper. The general distribution of *P. concinna* has been stated in Komarov's (17) Flora U.S.S.R. In the latter work, a sterile hybrid, *P. algida* × *P. concinna*, was listed and was said to occur infrequently with both species.

(14) *Pleuropogon sabinei* R.Br.

One collection was hexaploid, $2n = 42$.

Voucher specimen: Franklin District: Cyt. No. 692, Frobisher Bay, Baffin Island, *J. A. Calder 2135*; $2n = 42$ det. on one plant.

Holmen (12) reported $2n = 40$ for this species. Porsild (22) has mapped the geographical range in his map No. 40.

(15) *Schizachne purpurascens* (Torr.) Swallen

One collection was diploid, $2n = 20$.

Voucher specimen: Ontario: Carleton Co.: Cyt. No. 1855, between Harwood Plains and Shirley Bay, *W. M. Bowden 124-55*; $2n = 20$ det. on one clone.

Boyle (2) and Tateoka (30) reported $2n = 20$ for this species.

(16) *Torreyochloa fernaldii* (Hitchc.) Church

Three collections were diploid, $2n = 14$.

Voucher specimens: Prince Edward Island: Queens Co.: Cyt. No. 1554, Wood Islands, *D. Erskine 1329*; $2n = 14$ det. on one plant. Quebec: Cyt. No. 1861, just east of Gatineau Park road, 3.3 miles north of Eardley, *W. M. Bowden & W. G. Dore 129-55*; $2n = 14$ det. on one clone. Ontario: Carleton Co.: Cyt. No. 1832, Mer Bleue, Borthwick Creek, draining peat bog, south of Blackburn Station, *W. G. Dore & W. M. Bowden 15355*; $2n = 14$ det. on one clone.

Church (5) described the genus *Torreyochloa* and reported $2n = 14$ for *T. fernaldii*. The status of this genus has been discussed by Clausen (7) and by Church (5, 6). The evidence appears to favor the conclusions of Church (6) that the genus *Torreyochloa* is morphologically and cytologically distinct.

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# DIFFERENTIAL RESPONSE OF CERTAIN BARLEY AND WHEAT VARIETIES TO HARDENING AND FREEZING DURING SPROUTING[1]

G. DANTUMA[2] AND J. E. ANDREWS[3]

## Abstract

A number of barley and wheat varieties from different geographical areas were tested for cold hardiness in the sprouted seed stage. The young plants were subjected to a hardening process for varying periods and at different temperatures. Under appropriate conditions of hardening and freezing nearly all of the varieties of winter wheat and winter barley showed a similar hardening pattern, that is, a relatively high degree of hardiness and ability to harden in young stages of germination (up to 1 week of growth at 1.5° C), a minimum hardiness at 2 or 3 weeks of hardening at 1.5° C, and a second maximum of hardiness at about 6 weeks of hardening at 1.5° C. Spring varieties and some West European winter wheats with low winter hardiness did not show the second maximum of hardiness after 6 weeks at 1.5° C. In general, additional hardening for 1 week at −4° C produced a striking increase in hardiness. For barley this increase was produced at all stages of development whereas for wheat there was a large increase only after 1 to 5 weeks of prehardening at 1.5° C. Hardening at −4° C for longer than 1 week did not produce additional hardiness. Possibilities of testing for cold hardiness at different growth stages of sprouted seeds under various conditions of hardening are discussed.

## Introduction

In the ample literature on winter hardiness and cold hardiness of plants, relatively little attention has been paid to the hardiness of young growth or sprouted seeds. In recent years in different countries results of experiments have been published in which the correlation between cold hardiness of sprouted or soaked seeds and that of older stages of growth was determined. Ivanoff (8) working with oats in the U.S.A. and Segeta (12) with wheat and rye in Czechoslovakia were able to distinguish between varieties by exposing soaked unhardened seeds to low temperatures. Andrews (1) in Canada, Grahl (5) in Germany, and Saltykovsky (11) in Russia described methods of testing for cold hardiness of wheat during sprouting. The techniques of growing the wheat, the hardening procedure, and the freezing temperatures used by these three authors varied considerably.

Andrews (2) showed that for wheat duration of hardening in sprouted seeds had a marked influence on cold hardiness. Maximum cold hardiness and best differentiation between varieties were obtained after 6 weeks of hardening at 1.5° C. Hardiness increased rapidly during the first 5 weeks and decreased rapidly after 7 weeks at 1.5° C. The degree of hardening also varied markedly with small changes in hardening temperature.

Hänsel (6) reported that the primary roots of wheat were more susceptible to cold than the coleoptile and determined further that the cold hardiness of

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Contribution from the Cereal Breeding Section, Canada Agriculture Research Station, Lethbridge, Alberta.
[2]Cereal Section, Foundation for Agricultural Plant Breeding, Wageningen, The Netherlands; and Postdoctorate Fellow, Lethbridge, 1958–59.
[3]Cereal Breeding Section, Research Station, Lethbridge.

these roots is greatly influenced by their stage of growth. In his study with French winter wheat varieties, Jonard (9) reported that there is little hardiness to cold in the germination stage and that the coleoptile is the most susceptible part of the plant.

Dantuma (3) tested a number of wheat varieties of different origin. In the West European varieties studied there was little similarity between the cold hardiness in the sprouted seed stage and the hardiness in older plants but this similarity did occur in a number of the southeast European varieties.

Many data, especially in the European literature, can be found on winter hardiness of late-seeded wheat, but most are contradictory.

The main purpose of this study was to investigate the response to hardening and freezing during sprouting of barley and wheat varieties from different geographic regions and to determine whether varieties from different regions react similarly or differently.

## Materials and Methods

The varieties used in this study include winter wheat and winter barley from different geographic regions; a spring barley, Trebi; and two spring wheats, Thatcher and C.A.N. 3842. The latter spring wheat has fairly good winter hardiness under West European conditions. It was not possible to use seed of all varieties produced at one location.

Seed of the two German barley varieties Dea and Atlas and of the Dutch variety Urania was produced at Wageningen, Holland; of the spring variety Trebi at Lethbridge, Alberta; and of Wong, Hudson, and Kenate at Ottawa, Ontario. The varieties Meimi, C.I. 7407/3, and Kearney were obtained from Columbia, Missouri. There are no comparable data on winter hardiness for all of these varieties. Weibe and Reid (14) report the average percentage survival of Trebi, Wong, Kenate, Hudson, Meime, and Kearney as 24.2, 60.0, 67.9, 69.3, 77.1, and 82.2, respectively. The Dutch "List of Varieties of Field Crops", in which a scale from 1 to 10 (from weak to hardy) is used, rates the winter hardiness of the three European varieties Dea, Urania, and Atlas as 4, 5, and 7 respectively.

The wheat varieties studied were from two general areas of production, North America and Western Europe. The North American group included Thatcher (spring), C.A.N. 3842* (spring), Elgin, Jones Fife, Kharkof C.I. 1442, Kharkov 22 M.C., and Minturki. The West European group included Cappelle* (France), Leda* (Belgium), Heine VII* (Germany), Merlin* (Germany), Criewener 192* (Germany), Ertus (Sweden), Virtus (Sweden), Varma (Finland), and Olympia (Finland). Seed of the varieties marked with an asterisk was produced at Wageningen, Holland, and that of the other varieties at Lethbridge, Alberta.

Tested under mild conditions in Holland and reported in the Dutch "List of Varieties of Field Crops", the relative winter hardiness of the varieties Cappelle, Leda, Heine VII, and Merlin, rated on a scale from 1 to 10 (from

weak to hardy), was 3.5, 7, 8, and 9, respectively. On the same scale the spring variety C.A.N. 3842 was rated 7.5. The other four European varieties had better winter hardiness when tested in Holland during the cold winters of 1953–54 and 1955–56 but comparative ratings for each variety are not available.

Two types of plastic containers with hinged lids, one $3\frac{1}{2} \times 7$ in. with 10 compartments and the other $4\frac{1}{2} \times 8$ in. with 18 compartments, were used in the tests. Ten cubic centimeters of dry vermiculite (finish aggregate grade) were placed in each compartment as a substrate for growth and, with a few exceptions that are recorded, 7 ml of water were added to each compartment. After placing 20 seeds per compartment on the surface of the moist vermiculite the plastic boxes were placed in polyethylene bags to avoid uneven evaporation around the lids.

In general, the boxes were kept first at room temperature, about 22° C, for 16 hours and then moved to a dark, controlled-temperature room maintained at $1.5° \pm 0.5°$ C.

After hardening for a certain period the material was placed for 8 or 16 hours in freezing chambers controlled at $-9°$ or $-14°$ C, as described for the different experiments. In some experiments an additional period of hardening at $-4°$ C was used prior to exposure to $-9°$ or $-14°$ C. The temperature of all rooms was subject to minor fluctuations of $\pm 0.5°$ C.

After removal from the freezing chamber the material was held for 16 hours at 1.5° C. Then the seeds or young plants were covered with a sand–vermiculite mixture and placed in a growth chamber at 15° C. A nutrient solution as described by Andrews (1) was added as necessary.

Survival counts were made 2 weeks after exposure to freezing conditions. Where there was considerable variation within varieties in the response of plants to the freezing treatment the seedlings were classified into three classes and a survival index was calculated as described by Andrews (1).

All percentage data were transformed by means of the angular transformation (angle = arc sin/percentage) before analysis of variance was applied, and all means reported herein are transformed means. To establish the significance of differences among means the 'new multiple range test' described by Duncan (4) was used.

In all tables where this test was applied any two means *not underscored* by the same line are *significantly different* and any two means *underscored* by the same line are *not significantly different* at the 5% level.

## Results (Barley)

Preliminary experiments were carried out to determine (1) the optimum amount of water for germination and growth and (2) the best freezing temperature and period of hardening for varietal and treatment differentiation.

To determine the effect on germination seven levels of moisture were used, namely, 4, 5, 6, 7, 8, 9, and 10 ml of water per box compartment. Each

treatment was replicated six times and the varieties were allowed to germinate for 5 weeks at 1.5° C. Seven and 8 ml of water produced maximum growth, 4 and 5 ml a much delayed growth, while 9 ml in most cases showed a less favorable germination and retardation of root development and 10 ml a greatly reduced percentage of germination. Consequently 7 ml of water on 10 cc vermiculite per box compartment were used in all later experiments.

To determine an appropriate freezing temperature the varieties used in the above experiment were exposed to low temperatures after the following two series of hardening treatments:

(A) hardened at 1.5°C for 1, 2, 3, 4, or 5 weeks; and

(B) hardened at 1.5°C for 1 week plus an additional 2, 3, or 4 weeks at −4° C.

Both series were grown in duplicate and one was exposed for 8 hours at −9° C and the other 8 hours at −14° C. The results for the former are given in Table I.

TABLE I

Effect of duration of hardening at 1.5° C, and at −4° C, after prehardening 1 week at 1.5° C on survival of winter barley when exposed to −9° C for 8 hours

| | Weeks of hardening at 1.5° C | | | | | Weeks of hardening at −4° C after prehardening for 1 week at 1.5° C | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 2 | 3 | 4 |
| Variety | % survival | | | | | | | |
| Kearney | 35 | 17 | 18 | 33 | 20 | 65 | 70 | 63 |
| Meimi | 25 | 9 | 3 | 16 | 29 | 68 | 63 | 71 |
| Hudson | 53 | 9 | 20 | 17 | 20 | 88 | 88 | 68 |
| Wong | 8 | 3 | 0 | 7 | 0 | 41 | 51 | 63 |
| Trebi | 35 | 7 | 5 | 1 | 0 | 80 | 68 | 64 |
| Means | 31 | 9 | 9 | 15 | 14 | 68 | 68 | 66 |

The treatments at −4° C did not show any damaging effect but gave a marked increase in hardening. Cold hardiness after 1 week of hardening at 1.5° C was higher than after 2 and 3 weeks; it increased again after 4 and 5 weeks of hardening. Maximum hardening may not have been attained with 5 weeks of hardening. At 4 weeks of hardening at 1.5° C the relative survival of varieties was similar to their field winter hardiness. There was little survival after exposure to −14° C for 8 hours. Consequently −9° C was selected as the more appropriate freezing temperature for subsequent experiments with barley.

In a third experiment nine varieties were compared, the following hardening treatments being used: 1, 2, 3, 4, 5, and 6 weeks at 1.5° C; and 2, 3, 4, and 5 weeks at 1.5° C with an additional week of hardening at −4° C. There were 20 seeds per variety and 10 replicates per treatment. After hardening, all treatments were exposed simultaneously to −9° C for 8 hours.

During hardening at 1.5° C, growth continued slowly. There was some variation in growth rate between and within varieties. On the average the

varieties reached the following stages of growth after 1, 2, 3, 4, 5, and 6 weeks hardening at 1.5° C.

| Weeks at 1.5° C | Growth stage |
|---|---|
| 1 | First root just visible |
| 2 | Primary roots 15–20 mm long |
| 3 | Coleoptiles 4–9 mm long |
| 4 | Coleoptiles 8–15 mm long |
| 5 | Coleoptiles 12–20 mm long |
| 6 | Coleoptiles 18–30 mm long |

The survival percentages are shown graphically in Fig. 1. As in the previous experiments, survival was greater for each variety after 1 week of hardening at 1.5° C than after 2 or 3 weeks and for most varieties survival increased again from the 4- to the 6-week stage. The spring variety Trebi did not show increased hardiness between 4 and 6 weeks at 1.5° C and the European varieties Dea and Urania showed only a slight increase. Under less severe freezing conditions the latter two varieties might have responded similarly to the other winter varieties.

As the survival percentages of most treatments were low, only the results for 6 weeks of hardening were analyzed for variety differences. There was a significant difference between varieties ($P < 0.01$, S.E. = 1.2). The transformed variety means with differences indicated by Duncan's multiple range test are given below.

| Trebi | Urania | Dea | Atlas | Kearney | C.I. 7407/3 | Meimi | Hudson | Kenate |
|---|---|---|---|---|---|---|---|---|
| 0 | 8.2 | 12.3 | 19.1 | 28.1 | 28.9 | 29.5 | 30.6 | 30.7 |

With additional hardening of 1 week at −4° C in most cases a much higher level of cold hardiness was reached than with hardening at 1.5° C only. Figure 2 shows the relative survival, after exposure to −9° C for 8 hours, of barley varieties hardened 2 weeks at 1.5° C as compared with those hardened for 1 week at 1.5° C followed by 1 week at −4° C.

The analysis of variance showed that there was a significant difference between treatments and varieties ($P < 0.01$) and a significant variety × treatment interaction ($P < 0.01$). The highest survival was obtained with hardening for 5 weeks at 1.5° C plus 1 week at −4° C. Survival means of varieties with 5 weeks of hardening are shown below in order of magnitude, with significant differences indicated by Duncan's multiple range test.

| Trebi | Urania | Kearney | Dea | C.I. 7407/3 | Atlas | Meimi | Kenate | Hudson |
|---|---|---|---|---|---|---|---|---|
| 26.9 | 48.5 | 60.0 | 60.3 | 61.9 | 62.1 | 64.0 | 68.2 | 72.0 |

Under both hardening conditions varieties could be ranked generally into the same groups as indicated by their known field performance. In survival the spring variety Trebi was lowest; the three European varieties Urania, Dea, and Atlas were intermediate; Kenate and Hudson (U.S.A.) and Meimi (Korea) were the highest. Differences between individual varieties were not

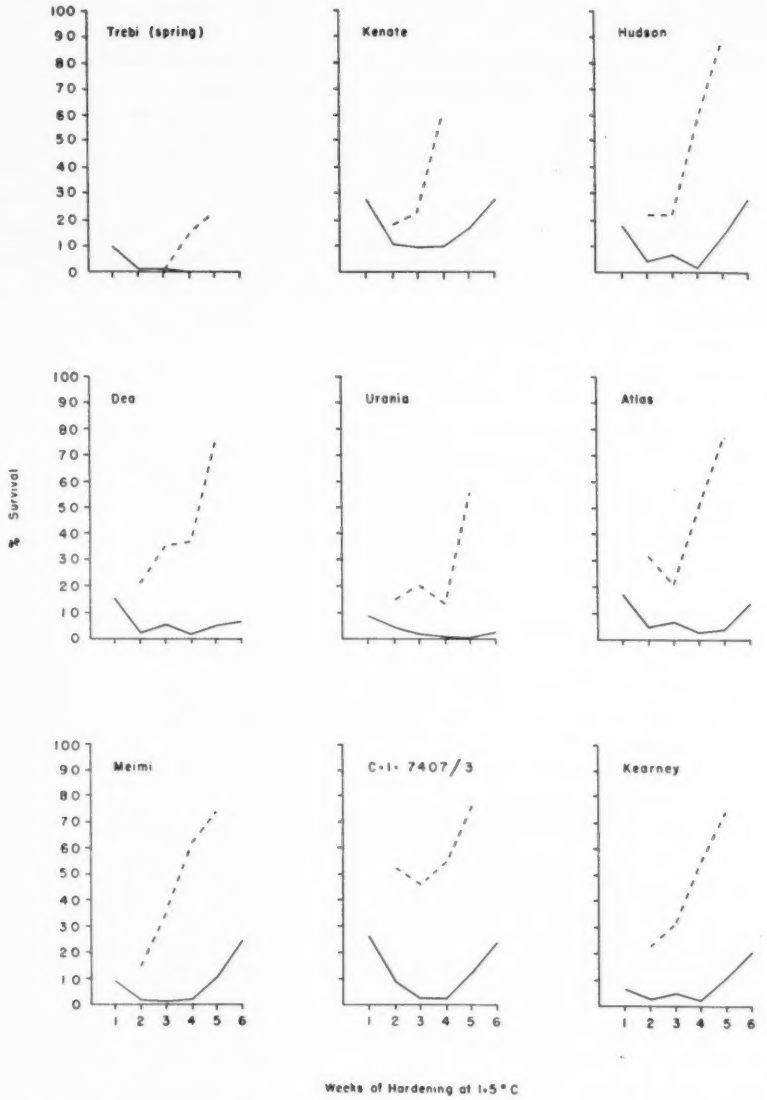


FIG. 1. Effect of duration of hardening at 1.5° C and of additional hardening at −4° C on the cold tolerance of one spring and eight winter barley varieties exposed to −9° C for 10 hours.

——— = hardened at 1.5° C.
— — — = hardened at 1.5° C followed by 1 week at −4° C.

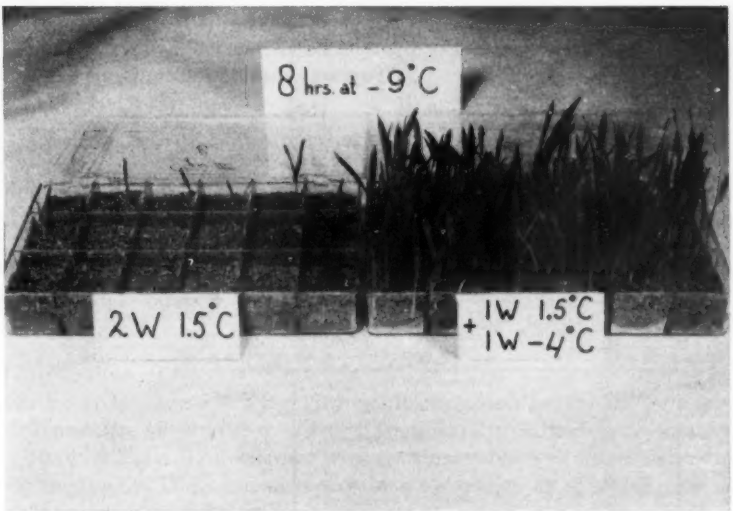


FIG. 2. Effect of additional hardening for 1 week at −4° C after prehardening 1 week at 1.5° C on the cold tolerance of barley varieties exposed to −9° C for 8 hours.

clearly established. The variety Kearney (U.S.A.) was lower in survival than expected, which may have resulted in part from the rather poor seed available. Because good seed of this variety was not available it was not tested further.

In a fourth experiment six of the above-mentioned varieties were compared in the following three series of treatments:

(A) hardened at 1.5° C for 1/7, 4/7, 1, 2, 4, 6, 8, and 10 weeks and exposed for 8 hours at −9° C;

(B) hardened at 1.5° C for 1, 3, 5, 7, and 9 weeks, plus 1 additional week at −4° C and exposed for 8 hours at −9° C; and

(C) hardened as in B but exposed for 16 hours at −9° C.

All treatments were exposed to freezing at the same time. The survival percentages for all treatments are shown graphically in Fig. 3. These results show clearly that there are two stages of growth at which barley varieties can develop cold hardiness. The first occurred during the first week of germination and hardening at 1.5° C and was followed by a point of minimum hardiness at 3 weeks of growth. Between 4 and 6 weeks under these conditions cold hardiness again increased rapidly and reached a maximum at about 6 weeks. As in the previous experiment, additional exposure to −4° C for 1 week after prehardening gave a striking increase in cold hardiness.

Analysis of variance of results from series A showed a significant difference between means for treatments ($P < 0.01$) and a significant treatment × variety interaction ($P < 0.01$, S.E. = 2.7). The spring variety Trebi showed high tolerance at the 1- and 4-day and 1-week treatments but low tolerance with longer durations of hardening. The other varieties reached a second

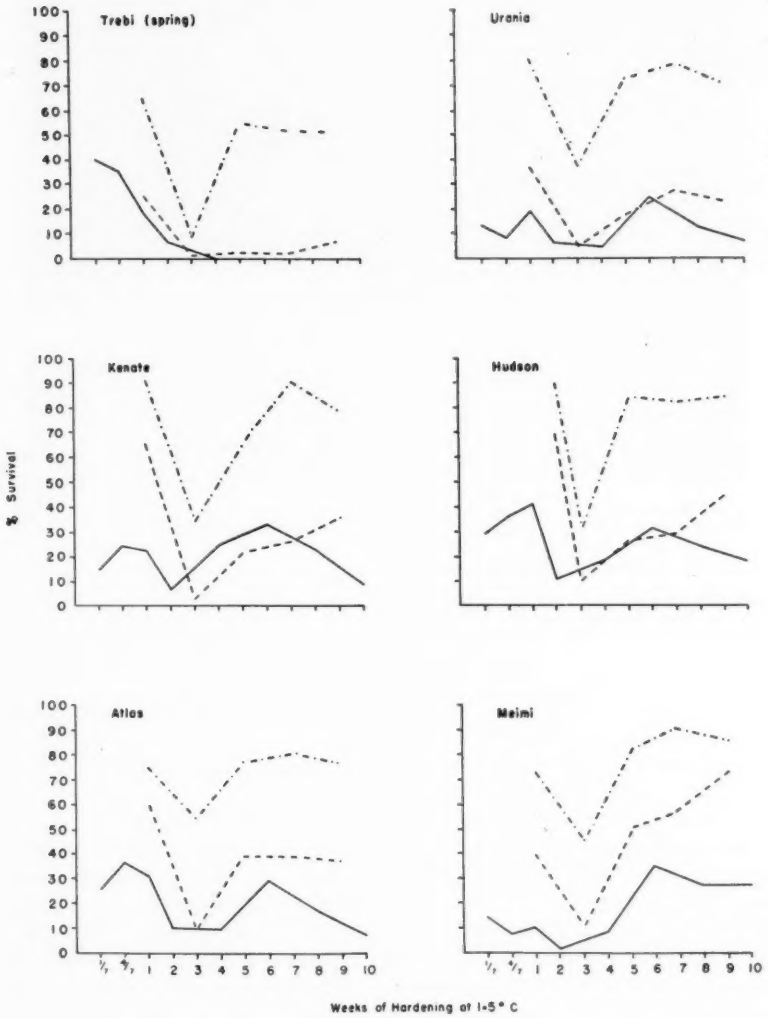


FIG. 3. Effect of duration of hardening at 1.5° C, and of additional hardening at −4° C after prehardening at 1.5° C on the cold tolerance of one spring and five winter barley varieties.

——— = hardened at 1.5° C, exposed to −9° C for 8 hours (series A).
—·—·— = hardened at 1.5° C, additional hardening 1 week at −4° C, exposed to −9° C for 8 hours (series B).
— — — = hardened at 1.5° C, additional hardening 1 week at −4° C, exposed to −9° C for 16 hours (series C).

peak of hardiness with 6 weeks of hardening at 1.5° C. The survival means of varieties at this stage are listed below in order of survival and differences are shown by Duncan's multiple range test.

| Trebi | Urania | Atlas | Hudson | Kenate | Meimi |
|---|---|---|---|---|---|
| 2.9 | 29.1 | 31.3 | 33.7 | 35.1 | 37.0 |

For series B there was a significant difference between means for treatments and varieties ($P < 0.01$, S.E. = 3.6). Highest average survival was obtained at 7 weeks of hardening. The survival means of varieties at this stage are listed in order of magnitude below and significant differences are indicated by the multiple range test.

| Trebi | Atlas | Urania | Hudson | Kenate | Meimi |
|---|---|---|---|---|---|
| 46.0 | 64.6 | 64.6 | 66.5 | 75.1 | 75.6 |

For series C there was a significant difference between means for varieties and treatments ($P < 0.01$) and a significant variety × treatment interaction ($P < 0.01$, S.E. = 2.6). Highest average survival was obtained with the 9-week treatment. The survival means of the varieties at this stage are listed in order of magnitude below and significant differences are indicated.

| Trebi | Urania | Kenate | Atlas | Hudson | Meimi |
|---|---|---|---|---|---|
| 10.6 | 27.9 | 36.7 | 36.8 | 42.4 | 59.6 |

Considering the results of the three series it is evident that the greatest differences were found when killing was moderately severe. With extra hardening at −4° C, freezing for 8 hours at 9° C (series B) was not sufficiently severe to reveal variety differences as well as the other two series. In both series A and series C Trebi was lowest and Urania second lowest in survival. Meimi was highest and the other three varieties were intermediate. This agrees with their field performance.

## Discussion (Barley)

Most varieties in all the experiments showed a maximum of cold hardiness at two stages of growth. The first maximum occurs in the youngest stage of germination just prior to the appearance of the first roots. In these experiments this stage was found in the first week of hardening at 1.5° C. This maximum is followed by a decrease in cold hardiness as growth progresses until a minimum was reached at about 3 weeks at 1.5° C. Between 4 and 6 weeks under these hardening conditions, cold hardiness increased to reach a maximum at about 6 weeks. After a longer period of hardening the cold hardiness decreased again, and this was probably associated with exhaustion of food reserves in the endosperm.

Supplementary hardening at −4° C for 1 week produced striking increases in cold hardiness in all treatments.

The young stages of germination (1 week of growth at 1.5° C) may show a high level of cold hardiness but do not appear to be very useful for testing

for cold hardiness. Most varieties showed a relatively high cold tolerance in this stage, even the spring variety Trebi. Small differences in stage of growth or rapidity of germination can cause considerable differences in cold tolerance that would make a satisfactory differentiation between varieties difficult or even impossible. Later stages of growth (5–7 weeks at 1.5° C) were more satisfactory for testing genetic differences and were probably less influenced by small differences in growth. The spring variety Trebi did not survive freezing when grown longer than 3 or 4 weeks at 1.5° C.

It is interesting to observe that under these treatments cold hardiness increased after 4 weeks of hardening to reach a maximum at about 6 weeks. By growing surviving plants of the varieties Atlas, Meimi, and C.I. 7407/3 to heading it appeared that these varieties were fully vernalized after 4–5 weeks of hardening at 1.5° C. In spite of complete vernalization cold hardiness appeared to increase. This is in contrast with results of experiments with older plants, from which it appears that vernalization reduced the hardiness of wheat and barley (7, 10, 13).

## Results (Wheat)

Two preliminary experiments were carried out to determine the best freezing temperature and period of hardening for varietal and treatment differentiation. There were 20 seeds per variety and six replicates per treatment.

In the first experiment the varieties Leda, Heine VII, Merlin, Criewener 192, Virtus, Varma, C.A.N. 3842, Elgin, Kharkof C.I. 1442, and Kharkov 22 M.C. were used with 2, 4, 6, and 8 weeks of hardening at 1.5° C. Exposure to −14° C for 8 hours resulted in survival of less than 10% for all the varieties except Kharkov 22 M.C. The survival percentages of this variety were 19, 19, 44, and 32, respectively, at the four durations of hardening.

In the second experiment the varieties Cappelle, Leda, Heine VII, Criewener 192, Virtus, Varma, C.A.N. 3842, Elgin, Jones Fife, and Kharkof C.I. 1442

TABLE II

Effect of duration of hardening at 1.5° C on survival of wheat varieties when exposed to −9° C for 8 hours

| | Weeks of hardening | | | |
|---|---|---|---|---|
| | 2 | 4 | 6 | 8 |
| Variety | % survival | | | |
| Cappelle | 29 | 21 | 11 | 12 |
| Leda | 23 | 4 | 21 | 6 |
| Heine VII | 12 | 27 | 26 | 0 |
| Criewener 192 | 27 | 47 | 27 | 28 |
| Virtus | 31 | 77 | 88 | 69 |
| Varma | 45 | 65 | 74 | 58 |
| C.A.N. C.I. 3842 | 19 | 42 | 49 | 24 |
| Elgin | 29 | 46 | 45 | 44 |
| Jones Fife | 30 | 43 | 77 | 59 |
| Kharkof 1442 | 31 | 59 | 64 | 44 |

were exposed at −9° C for 8 hours after hardening as in the first experiment. Results are given in Table II. With the exception of Cappelle and Leda, the hardening pattern of the varieties was more or less the same as that described by Andrews (2), namely, a low degree of hardiness at 2 and 8 weeks of hardening and a higher degree at 4 and 6 weeks of hardening, with a maximum for most varieties at 6 weeks. As a differentiating temperature, particularly between the hardier varieties, exposure to −9° C was not sufficiently severe. Although −14° C resulted in relatively low survival for most of the varieties in the experiment reported above, it was used again in subsequent experiments and, as will be seen from the results obtained, proved satisfactory. Facilities for using an intermediate freezing temperature were not available.

The striking increase in hardiness after extra hardening at −4° C with barley was a motive to include some variation in hardening temperatures in a third wheat experiment. Herein the varieties Kharkov 22 M.C., Minturki, Virtus, Ertus, Olympia, and Varma were compared in the following series of hardening treatments:

(A) hardened at 1.5° C for 2, 4, 6, 8, or 10 weeks;
(B) hardened at 5° C for 1 week plus additional hardening of 1, 3, 5, 7, or 9 weeks at 1.5° C; and
(C) hardened for 2, 4, 6, 8, or 10 weeks, the first half of each hardening period being at 1.5° C and the second half at −4° C.

There were 20 seeds per variety and six replicates per treatment. The three series were exposed simultaneously to −14° C for 8 hours. As an indication of the stage of growth, the coleoptile lengths of Kharkov 22 M.C. in the different hardening treatments are given in Table III. The survival percentages in all treatments are shown graphically in Fig. 4.

In general, the level of hardiness of series B was higher at 2 weeks than that of series A, and it then dropped below that of series A. Series B reached a maximum hardiness sooner than series A but at a lower level. This apparently resulted from the more rapid growth during the 1 week at 5° C.

TABLE III

Effect of duration of hardening and hardening temperature upon coleoptile lengths of Kharkov 22 M.C. winter wheat

| | Hardening treatment* | | |
|---|---|---|---|
| | A | B | C |
| Weeks of hardening | Coleoptile length, mm | | |
| 2 | 2 | 5 | — |
| 4 | 6 | 12 | 2 |
| 6 | 19 | 28 | 5 |
| 8 | 35 | 49 | 8 |
| 10 | 52 | 65 | 12 |

*A = hardened at 1.5° C for 2, 4, 6, 8, or 10 weeks.
B = hardened at 5° C for 1 week plus additional hardening of 1, 3, 5, 7, or 9 weeks at 1.5° C.
C = hardened for 2, 4, 6, 8, or 10 weeks of which the first half of the hardening period was at 1.5° C and the second half at −4° C.

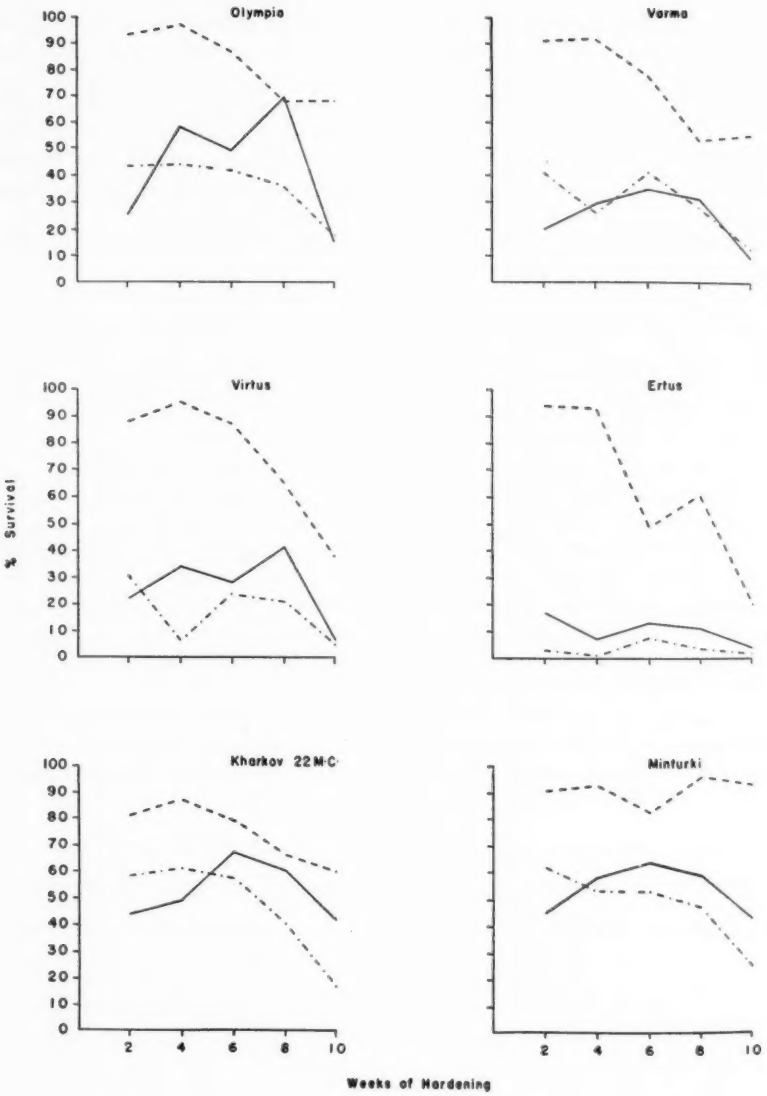


FIG. 4. Effect of different hardening treatments on the cold tolerance of six winter wheat varieties exposed to −14° C for 8 hours.
——— = hardening at 1.5° C (series A).
—·—·— = 1 week at 5° C and remainder of the hardening period at 1.5° C (series B).
— — — = first half of hardening period at 1.5° C and second half at −4° C (series C).

There was a large increase in hardiness after additional hardening at −4° C, especially at the younger stages of germination or with shorter durations of hardening (2–4 weeks). The maximum here was reached after 2 weeks at 1.5° C plus 2 weeks at −4° C (4 weeks total).

The analysis of variance showed a significant difference between varieties and treatments and a significant variety × treatment interaction for all three series (S.E. for series A, B, and C were 3.7, 2.6, and 4.7 respectively). The mean survival percentages of varieties with 6 weeks of hardening at 1.5° C are shown in order of magnitude in the following table, with significant differences indicated by the multiple range test.

| Ertus | Virtus | Varma | Olympia | Minturki | Kharkov 22 M.C. |
|---|---|---|---|---|---|
| 21.3 | 30.3 | 36.0 | 44.4 | 53.0 | 55.2 |

The fourth experiment was similar to the second but with more replication and two additional hardening treatments. It included the nine varieties, Cappelle, Leda, Merlin, Criewener 192, Ertus, Virtus, Elgin, Kharkof C.I. 1442, and Minturki, and six hardening treatments of 1, 2, 4, 6, 8, and 10 weeks' duration at 1.5° C. After hardening all were exposed to −9° C for 8 hours. There were 20 seeds per variety and 10 replicates per treatment. Compared with the results of the second experiment, the survival percentages in this experiment were high, as shown in Table IV, and generally were unsuitable for variety differentiation. This difference in plant survival between the two experiments may be explained by the difference in hardening temperature. During the hardening period the temperature of the room dropped to about 0.5° C and it is apparent that this difference in temperature caused a higher degree of hardiness. Some varieties, however, showed clearly two maxima of hardiness, the first one at a young stage of germination (1-week treatment) and a second one at about 6 weeks of hardening. With the higher degree of hardening the freezing temperature used was not sufficiently low to cause differentiation between all varieties and treatments.

TABLE IV

Effect of duration of hardening at 1.5° C on survival of wheat varieties when exposed to −9° C for 8 hours

| | Weeks of hardening | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 4 | 6 | 8 | 10 |
| Variety | % survival | | | | | |
| Cappelle | 88 | 73 | 74 | 70 | 81 | 65 |
| Leda | 65 | 60 | 59 | 74 | 62 | 54 |
| Merlin | 31 | 31 | 58 | 82 | 74 | 61 |
| Criewener 192 | 78 | 40 | 87 | 92 | 65 | 34 |
| Ertus | 62 | 34 | 73 | 92 | 86 | 78 |
| Virtus | 73 | 59 | 87 | 94 | 86 | 87 |
| Elgin | 65 | 52 | 74 | 81 | 78 | 71 |
| Kharkof 1442 | 83 | 58 | 74 | 69 | 67 | 34 |
| Minturki | 76 | 92 | 92 | 93 | 93 | 87 |

Nine varieties, Thatcher, Leda, C.A.N. 3842, Merlin, Elgin, Criewener 192, Kharkof C.I. 1442, Virtus, and Kharkov 22 M.C., ranging from low to high for winter hardiness, were compared in a fifth experiment involving two different hardening series listed below:

(A) hardened for 1, 2, 4, 6, 8, and 10 weeks at 1.5° C; and

(B) hardened for 1, 3, 5, 7, and 9 weeks at 1.5° C followed by 1 week at −4° C.

There were 20 seeds per variety and 10 replicates per treatment and all treatments were exposed simultaneously to −14° C for 8 hours. The survival percentages for all treatments are shown graphically in Fig. 5.

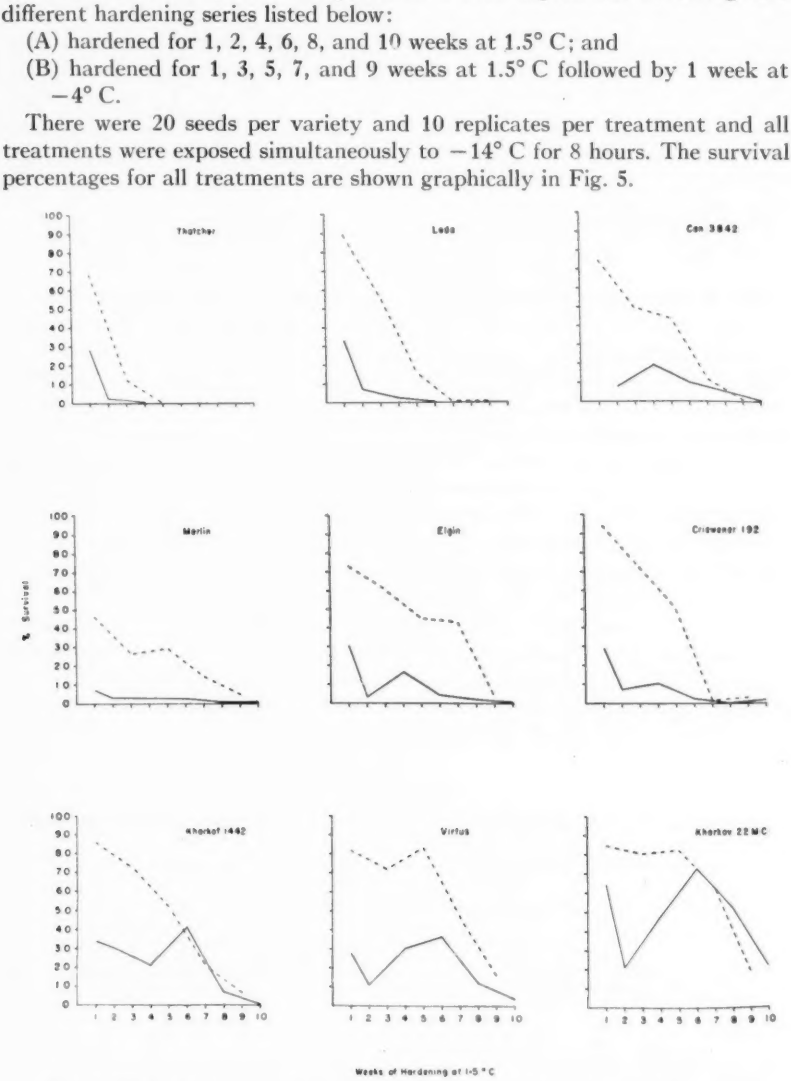


FIG. 5. Effect of duration of hardening at 1.5° C and additional hardening at −4° C after prehardening at 1.5° C on the cold tolerance of nine wheat varieties exposed to −14° C for 8 hours.

——— = hardened at 1.5° C (series A).

— — — = hardened at 1.5° C plus 1 week at −4° C (series B).

In series A the spring variety Thatcher showed a relatively high degree of hardiness in the young stage of germination but this decreased rapidly at the older stages. This was similar to the reaction of the spring variety Trebi in the barley experiments. The winter varieties Leda and Merlin under these more severe freezing conditions showed nearly the same pattern. The other winter varieties showed a second maximum of hardiness after 4 or 6 weeks of hardening at 1.5° C. This increased hardiness with 1 week of extra hardening at −4° C was striking in the younger stages of growth but there was little increase in the older stages. This is in contrast to the results obtained in the barley experiments.

The analysis of variance showed a significant difference between varieties and treatments and a significant variety × treatment interaction for both series (S.E. = 2.5 and 3.3 respectively).

The survival means of varieties with 6 weeks of hardening at 1.5° C are shown in order of magnitude below with significant differences indicated by the multiple range test.

| Thatcher | Leda | Criewener 192 | Merlin | Elgin | C.A.N. 3842 | Virtus | Kharkof C.I. 1442 | Kharkov 22 M.C. |
|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 6.4 | 7.0 | 10.1 | 18.0 | 36.1 | 39.8 | 59.4 |

The variety C.A.N. 3842 showed a higher level of cold hardiness than the more winter hardy European varieties Merlin and Criewener 192, which agrees with results obtained by Dantuma (3).

At the point of maximum hardiness after additional hardening at −4° C, that is, after 1 week at 1.5° C plus 1 week at −4° C, there were no significant differences between varieties. The survival means of varieties with 5 weeks of hardening at 1.5° C plus 1 week at −4° C are given below with significant differences indicated by the multiple range test.

| Thatcher | Leda | Merlin | C.A.N. 3842 | Elgin | Criewener 192 | Kharkof 1442 | Kharkov 22 M.C. | Virtus |
|---|---|---|---|---|---|---|---|---|
| 1.3 | 18.3 | 31.8 | 41.4 | 41.4 | 45.0 | 46.4 | 66.4 | 69.1 |

Again, the survival percentage of C.A.N. 3842 was higher than expected when compared with the winter hardiness of the variety Merlin. Under these conditions the European varieties Leda, Merlin, and Criewener 192 were ranked as expected from their field survival. Virtus showed a level of hardiness in this treatment higher than expected as compared with Kharkov 22 M.C.

To study the effect of a longer duration of hardening at −4° C, five varieties, Elgin, Virtus, Olympia, Minturki, and Kharkov 22 M.C., were compared in a sixth experiment with the following treatments:

(A) hardened for 1, 3, 5, 7, or 9 weeks at 1.5° C;

(B) hardened as in A, followed by 1 additional week at −4° C; and

(C) hardened as in A, followed by 3 additional weeks at −4° C.

All treatments, in 10 replicates of 20 seeds per variety, were exposed to −14° C for 8 hours at the same time. The survival percentages for all treatments are shown graphically in Fig. 6. The results show clearly that there is

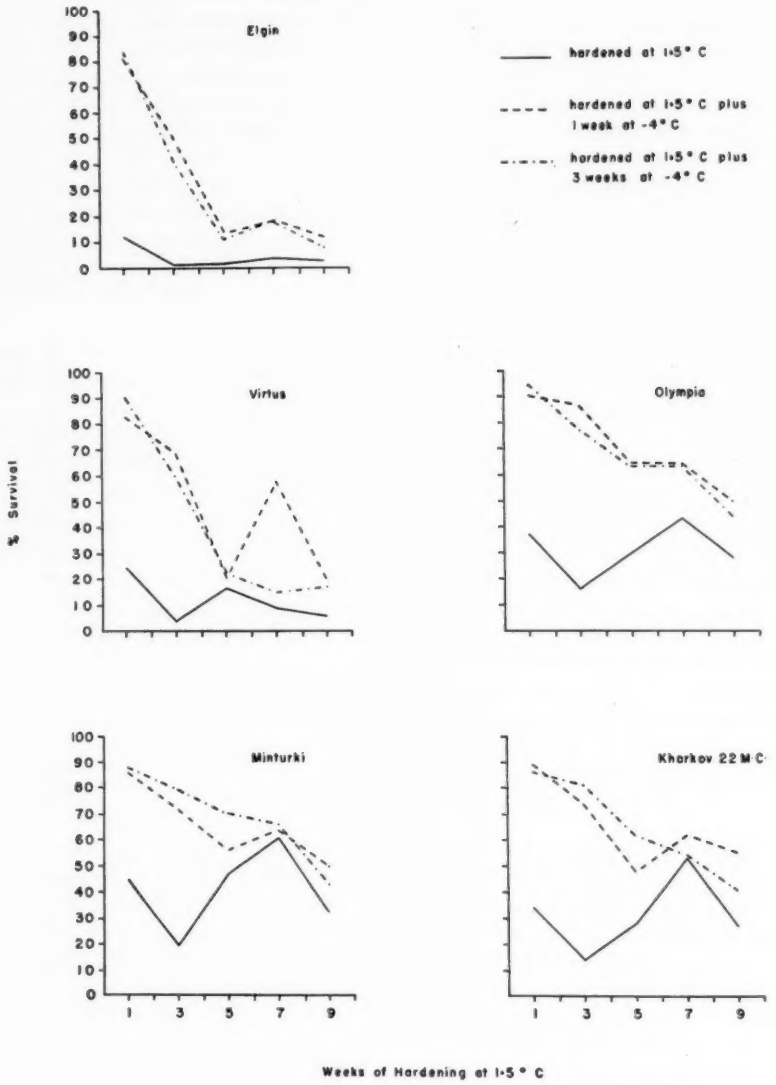
Elgin
hardened at 1·5° C
hardened at 1·5° C plus 1 week at -4° C
hardened at 1·5° C plus 3 weeks at -4° C
Virtus
Olympia
Minturki
Kharkov 22 M C
% Survival
Weeks of Hardening at 1·5° C

FIG. 6. Effect of duration of hardening at 1.5° C and additional hardening at −4° C after prehardening at 1.5° C on the cold tolerance of five winter wheat varieties exposed to −14° C for 8 hours.

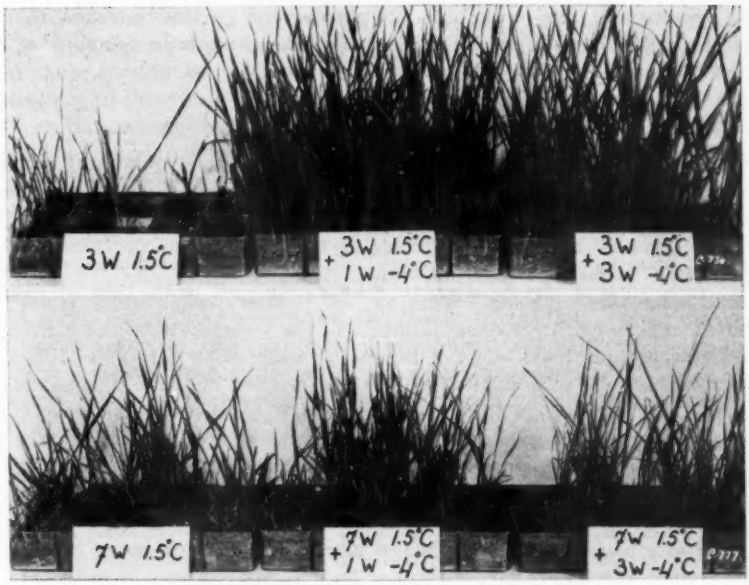


FIG. 7. Differential effect of additional hardening at −4° C after growth and pre-hardening for 3 as compared with 7 weeks at 1.5° C on the cold tolerance of wheat exposed to −14° C for 8 hours.

not much difference between the treatments B and C with the exception of the unexplainable difference for the variety Virtus at 7 weeks of hardening.

As in the foregoing experiment, there was a large increase in hardiness due to the additional hardening treatment of 1 week at −4° C in the younger stages of growth but only a small difference at the older stages. A hardening period longer than 1 week at −4° C did not increase hardiness. This is illustrated in Fig. 7.

The analysis of variance of results from series A and C showed a significant difference between means for treatments and varieties and a significant treatment × variety interaction (S.E. for each series 2.0 and 2.8 respectively).

Mean survival of varieties and significant differences by the multiple range test with 7 weeks of hardening at 1.5° C are given below.

| Elgin | Virtus | Olympia | Kharkov 22 M.C. | Minturki |
|---|---|---|---|---|
| 8.8 | 16.9 | 40.8 | 47.0 | 51.5 |

After 7 weeks of hardening at 1.5° C plus 3 weeks additional hardening at −4° C, the mean survival percentages of varieties and significant differences by the multiple range test were as follows.

| Virtus | Elgin | Kharkov 22 M.C. | Olympia | Minturki |
|---|---|---|---|---|
| 20.4 | 23.7 | 48.0 | 53.0 | 54.8 |

In general, the less hardy varieties showed the greatest response to the additional hardening and this resulted in some changes in the ranking of varieties.

## Discussion (Wheat)

Like barley, nearly all the winter wheat varieties under appropriate conditions of hardening and freezing show a similar hardening pattern, that is, a relatively high degree of hardiness in young stages of germination, a minimum hardiness at 2 or 3 weeks of hardening at 1.5° C, and then an increase to a maximum at about 6 weeks of hardening.

Additional hardening for 1 week at −4° C after prehardening at 1.5° C resulted in striking increases in hardiness in the youngest stages of germination. However, hardiness was not increased further with durations of longer than 1 week at −4° C. Compared with barley the effect of additional hardening at −4° C at older stages (6 or more weeks at 1.5° C) was small, especially with the hardiest varieties. In general, at this stage the less hardy varieties showed the greatest response to additional hardening at −4° C. This resulted in differences in ranking of varieties when tested after this additional hardening as compared with hardening at 1.5° C alone.

The spring variety Thatcher showed a relatively high degree of hardiness in the youngest stages of germination and not a second maximum as shown by most of the winter varieties tested. Some of the less hardy winter varieties, especially Cappelle, also did not follow the general hardening pattern described above. Where freezing was not severe, this variety showed nearly the same hardiness in all stages (Table IV) but without a minimum at 2 or 3 weeks' hardening at 1.5° C. Under more severe conditions many of the European varieties with marked differences in winter hardiness showed a low level of survival at all stages. Some of these varieties may resemble more or less the spring wheats in their hardening pattern or possibly the freezing temperature may have been too severe to reveal treatment differences.

As with the barley varieties, most wheat varieties have a relatively high degree of hardiness just after the seeds start to sprout (1 week at 1.5° C), but this does not appear to be a useful stage for testing for cold hardiness. Uniform results would be difficult to obtain because this stage of hardiness is apparently passed through rapidly and considerable change in degree of hardiness may result from small differences in stage of germination. Furthermore, all varieties including the spring variety Thatcher had a relatively high and similar level of hardiness at this stage. Although the use of −4° C as an additional hardening temperature resulted in a large increase in hardiness of all varieties, particularly with less than 5 weeks of prehardening at 1.5° C, a good differentiation between varieties also was not obtained under this hardening condition with the freezing temperature used. It is of course possible that lower freezing temperatures may have given different results.

Best differentiation was obtained with 5 to 7 weeks of hardening at 1.5° C.

As reported previously by Andrews (2), it is also evident that small differences in hardening duration, hardening temperature, and stages of germination cause considerable differences in cold hardiness. Varieties may respond differently to these changes, although in general they follow the same pattern. Differentiation between varieties is dependent upon the use of an appropriate freezing temperature for the degree of hardening that has occurred. Under the hardening and freezing conditions used in these studies, the varieties could be separated into three general groups. The North American varieties Kharkov 22 M.C. and Minturki were the most hardy, the Scandinavian varieties were intermediate, and the West European varieties were the least hardy. In most cases, the varieties were ranked in about the same order as for field survival but there were a few exceptions, particularly among the West European varieties. This may have resulted from failure to select the most suitable freezing temperature or it may mean that survival in the field in Western Europe is governed by factors other than those associated with this testing procedure.

## Acknowledgments

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# THE DEVELOPMENT OF FOLIAR SYMPTOMS AND THE POSSIBLE CAUSE AND ORIGIN OF WHITE PINE NEEDLE BLIGHT[1]

S. N. LINZON[2]

## Abstract

Needle blight of eastern white pine (*Pinus strobus* L.) is characterized by an orange-red discoloration of the distal portions of current year needles. At Chalk River, Ontario, 600 seedlings and saplings of white pine were examined repeatedly throughout the 1957 and 1958 growing seasons for the appearance and development of needle blight symptoms. The first of these were faint pinkish spots on the stomata-bearing faces of needles in semimature (about four-week-old) tissue. These developed rapidly into orange-red bands which within a few days spread to the needle tips. Mature tissue was not susceptible to attack, so lesions which developed subsequently were always proximal, on younger tissues derived from basal meristems. Attacks which occurred in the early summer killed only limited areas at the tips of needles, whereas those that occurred when the needles were nearly full-grown involved most of their length. Needle blight incidence was confined to a few major outbreaks during one season and in each of these many trees developed typical symptoms at essentially the same time. Each of these major outbreaks of the disease occurred after 1 or more days of wet weather which was followed suddenly by a continuous sunny period. No microorganisms were isolated from tissues displaying the initial needle blight symptoms.

The data with respect to the nature and occurrence of needle blight are believed to suggest that susceptibility to the unfavorable conditions which incite it is inherent in the individual and that differences in response among members of a local population depend on variations in susceptibility, rather than on a varying local predisposition among uniformly susceptible individuals. The blight is initiated in semimature leaf tissues only but then spreads distally throughout adjacent, more mature tissues with a similar pattern of breakdown.

## Introduction

The most conspicuous symptom of needle blight is the injured foliage. The distal portions of the needles of the current year are killed and develop a bright orange-red (7.5 R 5/10 according to the Munsell system (1)) color. Diseased trees may also display small leaves and twigs, premature defoliation, reduced height and diameter growth, and poorly developed root systems (5, 16, 19, 20) (Figs. 1–4).

Foliage injury on white pine has been reported throughout the range of the species and has been discussed under a variety of names. These names include blight (6, 7, 14, 23), tip burn (4), short needle (14), dwarf needle (12), chlorotic dwarf (22), sun scorch (21), sand burn (3), yellow dwarf blight (5), red needle blight (5), and needle blight (2, 5, 8, 9, 10, 11, 13, 15, 16, 17, 18, 19, 20, 24).

Campana (5) in the New England States differentiated between two foliage injuries of white pine which have often been confused in the past, and gave them the names red needle blight and yellow dwarf blight. Red needle blight is the typical needle blight injury found in the Province of Ontario, while yellow dwarf blight is referred to as chlorosis. Chlorosis affects white pine

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Contribution No. 577, Forest Biology Division, Research Branch, Department of Agriculture, Ottawa, Canada.
[2]Regional Research Laboratory (Forest Biology), Maple, Ontario.

foliage of all ages with the discoloration appearing yellowish-green (7.5 Y 8/8 according to the Munsell system (1)).

Field investigations to determine the nature and cause of white pine needle blight have been conducted mainly at the Petawawa Forest Experiment Station, Chalk River, Ontario. The station provides both planted and naturally reproduced pine stands of several ages, and is remote from any smelter fumes which might cause similar markings on foliage (18).

## Materials and Methods

On a 1/2-acre sample plot of natural tree growth, 600 white pine seedlings and saplings, 1 ft and over in height, were tagged in 1957. The average age and height of these trees was 13 years and 4 ft, respectively. These trees were examined almost daily throughout the growing seasons of 1957 and 1958 for the appearance and development of the foliar symptoms of needle blight. The severity of needle blight in the crowns of the tagged white pines was determined from an intensity scale prepared for this purpose which is outlined in Table I. The foliage growth of a selected group of comparable needle-blighted and healthy trees on the plot was measured weekly.

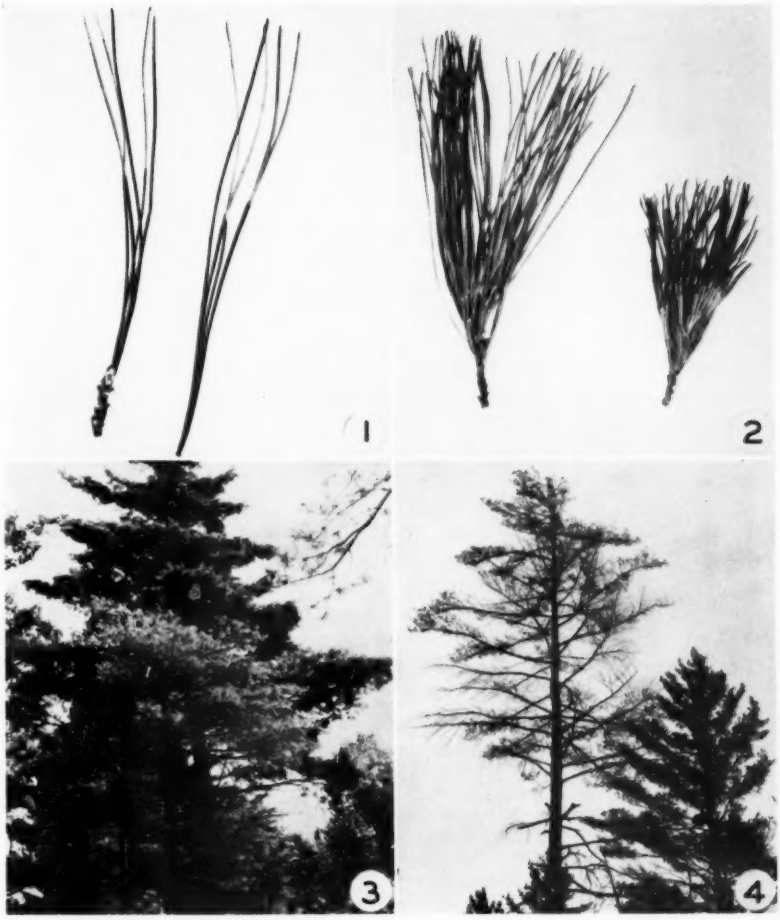
TABLE I

White pine needle blight severity classification
(Based on an orange-red discoloration of the current year foliage)

| Character | Factor | Value |
|---|---|---|
| Proportion of crown affected | Nil | 0 |
| | Less than 1/3 | 1 |
| | 1/3 – 2/3 | 2 |
| | More than 2/3 | 3 |
| Proportion of needle discolored | Nil | 0 |
| | Less than 1/3 | 1 |
| | 1/3 – 2/3 | 2 |
| | More than 2/3 | 3 |
| Length of new needles | 3 in. or more | 0 |
| | 2–3 in. | 1 |
| | 1–2 in. | 2 |
| | Less than 1 in. | 3 |
| Length of new twigs | 5 in. or more | 0 |
| | 3–5 in. | 1 |
| | 1–3 in. | 2 |
| | Less than 1 in. | 3 |
| Premature defoliation of middle-aged and oldest needles | None | 0 |
| | Oldest foliage partly lacking | 1 |
| | Oldest foliage lacking | 2 |
| | Oldest foliage lacking and middle-aged foliage partly lacking | 3 |

When the values for these five characters are added together a total numerical rating is obtained which expresses the condition of the tree.

| | |
|---|---|
| 0 | — Apparently healthy |
| 1– 5 | — Mild needle blight |
| 6–10 | — Medium needle blight |
| 11–14 | — Severe needle blight |
| 15 | — Almost dead |




FIGS. 1 to 4. Needle blight of white pine.*
FIG. 1. Individual fascicles with injured needles.
FIG. 2. Foliage clusters from a healthy and a needle-blighted tree.
FIG. 3. Diseased tree in front of a healthy tree.
FIG. 4. Severely needle-blighted tree in foreground.

*If photographs were in color, the injured leaf areas would appear bright orange-red in contrast to the normal green of the uninjured leaf areas.

Data on the atmospheric conditions which occurred immediately prior to and during each major outbreak of needle blight in the area were taken from detailed daily weather records of the Canada Department of Northern Affairs and National Resources station at Chalk River.

Tissue from both blighted and healthy foliage at various stages of development was plated on various standard media.

## Observations and Results

White pine foliage consists of acicular, flexible, green needles with acute apices and serrulate margins. They are triangular in cross section and possess longitudinal furrows of stomata on their ventral faces. The needles are borne in fascicles of five, spirally arranged on the shoot, have basal meristems, and attain lengths of approximately 3 in. during one growing season. During the summer, trees bear needles produced in as many as three different years.

In 1957, at Chalk River, needle blight symptoms first appeared on white pine foliage of the current year on July 1. The initial injury occurred as faint, pinkish spots on the stomata-bearing faces of needles in semimature (about four-week-old) tissue. The spots developed into orange-red bands which spread to the older needle tissue in the apex within a few days. The needles continued to elongate from the basal meristems for another 3 weeks, whereas the apical lesions did not increase in length proximally.

A number of trees were affected again on July 29, 1957, when comparable spots appeared on the stomatal faces of the needles and developed into similar colored bands. The tissue affected was farther from the apices, and some green tissue was left between the two injured areas. It was apparent that needle tissue of approximately the same stage of maturity was affected in both attacks. In the following weeks the green areas between the two injured portions became reddened as the second injury spread distally.

On one affected tree, a third attack occurred about two weeks after the second, the injury appearing near the base of the needles. The basal needle

TABLE II

The occurrence of the foliar symptoms of needle blight on 600 tagged white pines on a sample plot at Chalk River in 1957

| | Number of trees | | | | |
|---|---|---|---|---|---|
| | Foliar symptoms first observed | | | Total, with needle blight | Percentage of trees with needle blight |
| Record date | First onset | Second onset | Third onset | | |
| June 26 | 0 | 0 | 0 | 0 | 0.0 |
| July 3 | 68 | 0 | 0 | 68 | 11.3 |
| 10 | 28 | 0 | 0 | 96 | 16.0 |
| 17 | 10 | 0 | 0 | 106 | 17.7 |
| 24 | 4 | 0 | 0 | 110 | 18.3 |
| 31 | 0 | 25 | 0 | 110 | 18.3 |
| Aug. 7 | 3 | 4 | 0 | 113 | 18.8 |
| 14 | 0 | 5 | 1 | 113 | 18.8 |
| 21 | 0 | 0 | 0 | 113 | 18.8 |
| 28 | 0 | 0 | 0 | 113 | 18.8 |
| Sept. 4 | 0 | 0 | 0 | 113 | 18.8 |

tissue at this time was approximately as mature as had been the needle areas previously affected.

The numbers of trees affected on the sample plot at Chalk River, as described above, are recorded in Table II at weekly intervals during the summer of 1957.

Weekly measurements showed that healthy trees made better foliage growth than diseased trees and that needle elongation continued for approximately two weeks longer in the former. Figure 5 illustrates the needle growth of a needle-blighted and a healthy tree at Chalk River in 1957. Although the same basal, medial, and apical fascicle on each tree was repeatedly measured throughout the growing season, the foliage growth in Fig. 5 is shown by the linear outline of one needle which represents the average growth of the five needles of the basal fascicle. The blighted leaf areas on the affected tree are shown in black.

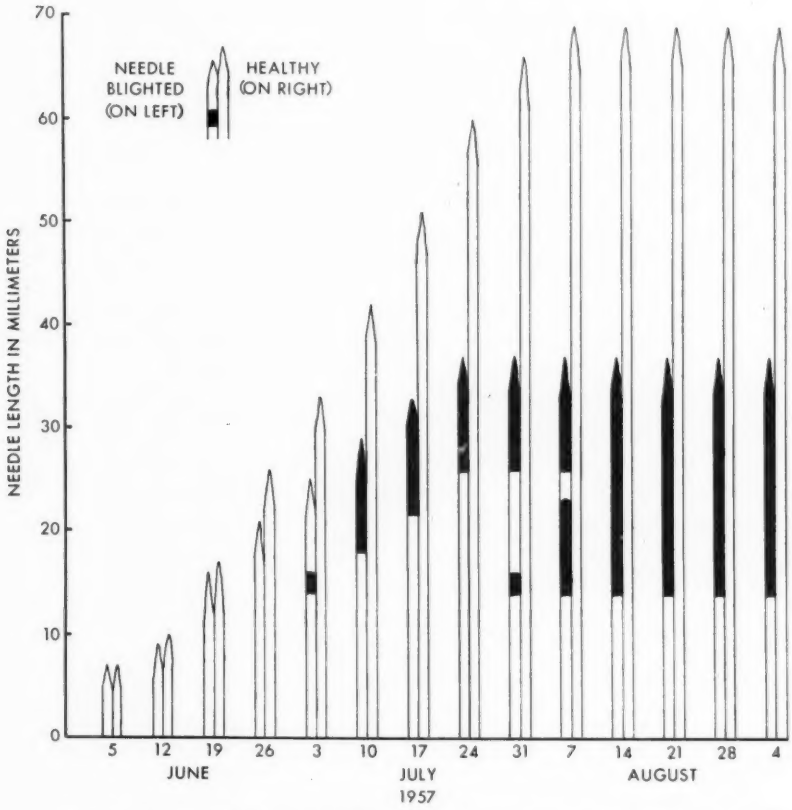



FIG. 5. Foliage growth of a needle-blighted and a healthy white pine tree at Chalk River in 1957. Symptom development shown on affected foliage.

In addition to the general pattern of needle injury which has been described, mottling and chlorosis occurred on some needles on affected trees in 1957. Thirty-three out of a total of 113 affected trees on the sample plot showed needle blight only on the upper fascicles of lateral branches, with the lower, shaded fascicles remaining green. None of these 33 trees experienced a second attack in 1957, and almost all were classified as being mildly blighted.

A classification of the disease incidence on the white pines located on the sample plot at Chalk River in September, 1957, is shown in Table III.

TABLE III

1957 classification of the disease incidence on white pines located on sample plot at Chalk River

| Tree condition | Total numerical value | Number of trees |
|---|---|---|
| Apparently healthy | 0 | 487 |
| Mild needle blight | 1 | 0 |
| | 2 | 5 |
| | 3 | 10 |
| | 4 | 24 |
| | 5 | 21 |
| Subtotal | | 60 |
| Medium needle blight | 6 | 10 |
| | 7 | 14 |
| | 8 | 16 |
| | 9 | 9 |
| | 10 | 0 |
| Subtotal | | 49 |
| Severe needle blight | 11 | 2 |
| | 12 | 1 |
| | 13 | 0 |
| | 14 | 1 |
| Subtotal | | 4 |
| Almost dead | 15 | 0 |
| Grand total | | 600 |

A total of 3.27 in. of rain fell at Chalk River on the last three days in June, 1957. On July 1 and 2 almost continuous sunshine occurred, and the foliar symptoms of needle blight appeared ubiquitously in the area. Data on the atmospheric conditions occurring immediately prior to and during this major outbreak of foliar symptoms were taken from the daily weather records of the Canada Department of Northern Affairs and National Resources and are shown in Table IV.

In 1958, the developmental history of the symptoms of needle blight at Chalk River was essentially the same as in 1957, the only difference being the time of symptom appearance. A major outbreak occurred on July 20, which was about three weeks later than in 1957. The needles on diseased trees were

TABLE IV

Daily weather* occurring immediately prior to and during major outbreak of the foliar symptoms of needle blight on white pine trees at Chalk River in 1957

| Date | Rain, inches | Relative humidity, percentage | Evaporation value | Maximum temperature, °F | Total sunshine, hours |
|---|---|---|---|---|---|
| June 28 | 0.78 | 100 | 14.0 | 72.5 | 0.0 |
| 29 | 1.86 | 95 | 0.0 | 69.0 | 0.0 |
| 30 | 0.63 | 85 | 15.0 | 62.0 | 0.0 |
| July 1 | 0.05 | 90 | + | 63.0 | 12.2 |
| 2 | 0.00 | 72 | 86.0 | 67.0 | 12.2 |

*Taken from records of Canada, Department of Northern Affairs and National Resources.

almost fully grown with the susceptible semimature tissue occurring farther from the apices, and thus more of the needle length was involved than was the case during the first onset in 1957.

A number of trees were affected again on September 8 in 1958 but this was too late for the injury to occur in mature, spring-shoot needles. In this case only needles on summer-shoots which originated from buds developed during the current season were affected, and the injury followed exactly the same pattern as described above.

Each major outbreak of the foliar symptoms of needle blight in 1958 occurred after 1 or 2 days of wet weather, which were followed immediately by a continuous sunny period.

Some additional observations were made on the relative amounts of available sunlight and the occurrence of needle blight symptoms on diseased trees. Symptoms were often found on trees along roads, on the edges of forest stands, in stand openings, and in cut-over areas. Also, in many cases only the top branches of a tree or only the upper fascicles of lateral branches of an affected tree were injured. Some diseased trees, or portions of a diseased tree, received more sunlight and thus were subject to more sudden injury at critical times than neighboring trees or other portions of the same diseased tree.

Although repeated attempts were made using various standard media no microorganisms were isolated from green needles prior to symptom appearance, or from initially injured orange-red leaf areas. Several fungi, apparently saprophytic, were obtained later from the killed leaf apices.

## Discussion

When this investigation was begun in 1957, it was believed that white pine needle blight affected the tips of needles first, and that this was followed by a progressive dieback (5, 7, 8, 9, 10, 11, 15, 20, 23, 24). It was also believed that an attack early in the summer would affect most of the length of the needles since it had a longer period of time to progress than an attack which occurred later in the summer (15).

However, our observations on the appearance and development of the foliar symptoms of white pine needle blight at Chalk River in 1957 and 1958 revealed that, if a tree was affected early in the summer, when the tips of the

needles possessed the susceptible stage of semimature tissue (about four weeks old), injury would occur at the tips, but no progressive dieback would ensue. More often, injury occurred at a later date, after the tissue in the tips had matured, and, when, because of basal meristematic activity, the susceptible semimature tissue was lower in the needle. The injury began at this point and spread into the older tissue in the apex. The lengths of discoloration on the current needles were shown to be the resultant of the maturity of the needle tissues and the times during the growing season when the different attacks occurred.

The 1957–58 observations were made on natural young white pine which allowed repeated close-up examination of their foliage throughout the growing seasons. In previous studies (15, 18) larger trees were examined, and the foliar symptoms were obvious only after the discoloration spread to the tips of the needles and were pronounced.

The foliage of most of the affected white pine in the area was injured at approximately the same time(s) during the summer. Also, it has been found that the annual incidence of the disease followed the same pattern on widely separated sample plots in the same local area (19). Thus, it was apparent that local weather conditions played a role in the appearance of the foliar symptoms on susceptible trees. Each major outbreak of the foliar symptoms at Chalk River in 1957 and 1958 occurred after a period of 1 or more days of wet weather which was immediately followed by a continuous sunny period.

Spaulding and Hansbrough (20) stated that "The white pine needle blight usually appears after prolonged periods of cloudy, moist weather which are followed abruptly by dry, hot days." Güssow (10) and Baldwin (2) have reported high incidences of needle blight in wet summers at Muskoka, Ontario, and in New Hampshire, U.S.A., respectively.

At Chalk River, cultures of green needles prior to symptom appearance and of initially injured, semimature needle tissue were sterile, although several apparently saprophytic fungi were obtained later from the killed leaf apices. Toole (23) failed to infect trees by inoculation with associated needle fungi or to arrest the disease by the use of fungicides. Faull (9) was unable to transmit the disease symptoms when he inoculated "healthy twigs of healthy trees with the juice from diseased foliage." Campana (5) was unable to induce needle blight in white pine seedlings after infecting the roots with *Corticium galactinum* (Fries) Burt. No relationship was found between needle blight and heartwood decay in the commercial portions of stems of white pine trees (17).

Why are some white pines susceptible to needle blight while neighboring trees growing on the same site under the same environmental conditions are resistant? It is suggested that the response of trees in exhibiting the foliar symptoms of needle blight when subjected to adverse climatic conditions is a heritable trait. The following considerations appear to the author to support the thesis that pines vary in their susceptibility to needle blight and that this reaction is controlled genetically.

1. Needle-blighted trees occurred over a wide range of environmental conditions. These included such contrasting sites as wet and dry, clay and sand, and flat and hilly, and such contrasting types as planted and naturally reproduced areas, dense and open stands, and pure and mixed stands (5, 13).

2. Diseased trees which displayed the foliar symptoms of needle blight in successive years were found growing with their crowns and roots intermixed with those of healthy trees, which were never observed to exhibit the symptoms of needle blight (19).

3. Previously affected trees which did not display the foliar symptoms in favorable years exhibited them again in years of high incidence in the area (19).

4. The annual occurrence of needle blight followed the same pattern on sample plots set out in replica in each of two kinds of plantations established for provenance studies (19). One plantation contained replicated blocks of white pine in which each different replication was established from seedlings obtained from different geographical locations. The other plantation contained replicated blocks of white pine in which each different replication was established from seed from a single mother tree. All mother trees supplying seed for these replications grew in the same locality.

5. The disease was demonstrated to be non-infectious (23) and non-communicable (9). At Chalk River, no microorganisms were cultured from needle tissue exhibiting initial stages of injury.

6. Dr. A. J. Riker[3] and Mr. B. W. Dance[4] have found that needle-blighted scions which were grafted on healthy stock exhibited the foliar symptoms annually thereafter, while the healthy stock remained unaffected. The reverse grafts were not attempted.

This paper presents further evidence that needle blight is non-parasitic in etiology. It is indicated that, with inherently susceptible trees, unfavorable environmental conditions induce the disease in semimature leaf tissues only. A breakdown once initiated in such tissues advances distally into more mature tissues which break down in a similar manner.

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# FUNGITOXICITY OF CHLORINATED HYDROCARBON INSECTICIDES IN RELATION TO WATER SOLUBILITY AND VAPOR PRESSURE[1]

LLOYD T. RICHARDSON AND D. M. MILLER

## Abstract

Fungitoxicity, in terms of inhibition of mycelial growth, was demonstrated with eight chlorinated hydrocarbon insecticides by the culture plate bio-assay method. All of the dosage–response curves thus derived were bimodal rather than linear. Evidence is presented to show that the shape of these curves is governed by two physical properties of the compounds: water solubility (which was determined experimentally) and vapor pressure. The concentration of insecticide in solution governs the initial rise of each dosage–response curve which is extended to a peak because of supersaturation and is followed by a decline as normal saturation is regained. The concentration of toxicant vapor in contact with the organism governs the second rise in the curve to a level dependent on the vapor pressure of the compound. Lindane has a high degree of fungitoxicity because of its relatively high water solubility. Heptachlor, chlordane, and aldrin are also highly toxic because of their vapor pressures. DDT, methoxychlor, dieldrin, and endrin are weakly fungitoxic because of both low water solubility and low vapor pressure.

## Introduction

In the course of an investigation of chemical seed treatments for the control of seed decay and pre-emergence damping-off due to soil-borne pathogenic fungi (7), it was found that certain insecticide–fungicide combinations provided better protection than fungicides alone even in the absence of insects. In an attempt to account for the beneficial effect of these insecticides, a study of their fungitoxicity in artificial culture was undertaken. Since toxicity of these chemicals to spores of various fungi could not be demonstrated in previous work (7, 12, 13), and since vegetative growth of the pathogens involved is essential for the development of the disease under natural conditions, inhibition of mycelial growth in culture was taken as a measure of fungitoxicity. An isolate of *Rhizoctonia solani* Kühn pathogenic to pea seedlings was selected as the test organism because of its rapid growth rate, uniform circular colonies on solid medium, and sensitivity to the chemicals tested. The investigation was confined to chlorinated hydrocarbon insecticides since several of this group are applied as seed and soil treatments in commercial practice.

## Methods

The insecticides were assayed by the commonly used plate culture technique. The test organism was grown on solid culture media containing a logarithmic dosage series of each insecticide. The required amount of chemical dissolved in 1 ml acetone was injected into 100 ml melted Czapek's agar cooled to 50° C.

[1]Manuscript received November 3, 1959.
Contribution No. 167, from Pesticide Research Institute, Canada Department of Agriculture, London, Ontario.

After brief shaking each lot of medium was poured into six replicate Petri dishes and allowed to solidify. A 4-mm inoculum disk cut from the margin of a 2-day plate culture was then inverted at the center of each plate.

To determine the extent to which inhibition of fungal growth by an insecticide under these conditions could be attributed to vapor of the chemical released at the surface of the medium, a two-layer culture plate was devised (see Fig. 1). Parallel series of these plates were used, one set having the chemical dosages in the upper inoculated layer of agar only, and the other set having chemical in the opposite layer only.

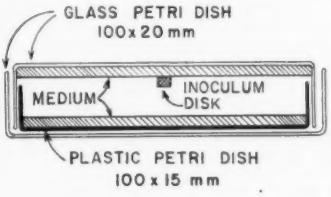


FIG. 1. Two-layered culture plate used to study inhibition of fungal growth by vapor from an insecticide.

Colony diameters were measured after incubation at 25° C for 3 days. The degree of inhibition of growth was calculated from the mean differences between treatments and controls as percentage of the latter. Dosage–response curves were derived by plotting the percentage inhibition on a probit scale against chemical concentration on a log scale.

Solubilities of most of the insecticides in water were determined by the following procedures. The insecticides[2] were first recrystallized repeatedly until a melting point unchanged by further recrystallization was obtained. The solvents used for this purpose were acetone in the case of endrin, methanol for chlordane, heptachlor, and lindane, and an equivolume mixture of ethanol and acetone for aldrin and dieldrin. Several crystals of each of these purified compounds were added to 2 liters of water contained in 3-liter glass-stoppered flasks. The flasks were shaken for several days in a water bath at a set temperature regulated to better than $\pm 0.1°$ C. Two 200-ml samples were then withdrawn from each flask and the insecticide content determined as outlined below. Similar determinations were made after further intervals of 3 to 4 days until saturation of the solution was indicated. This procedure was repeated at various temperatures.

The insecticides present in the samples were extracted with two portions of Eastman Kodak spectro grade 2,2,4-trimethylpentane (TMP). In the case of aldrin, endrin, dieldrin, and methoxychlor, these extracts were made up to 10 ml with TMP and their ultraviolet spectra recorded on a Beckman DK-1 spectrophotometer. Comparison of these spectra with those of standard solutions of the insecticides in TMP at approximately the same concentration

[2]Commercial stock except in case of gamma chlordane, a crystalline sample of which was supplied by Velsicol Corporation.

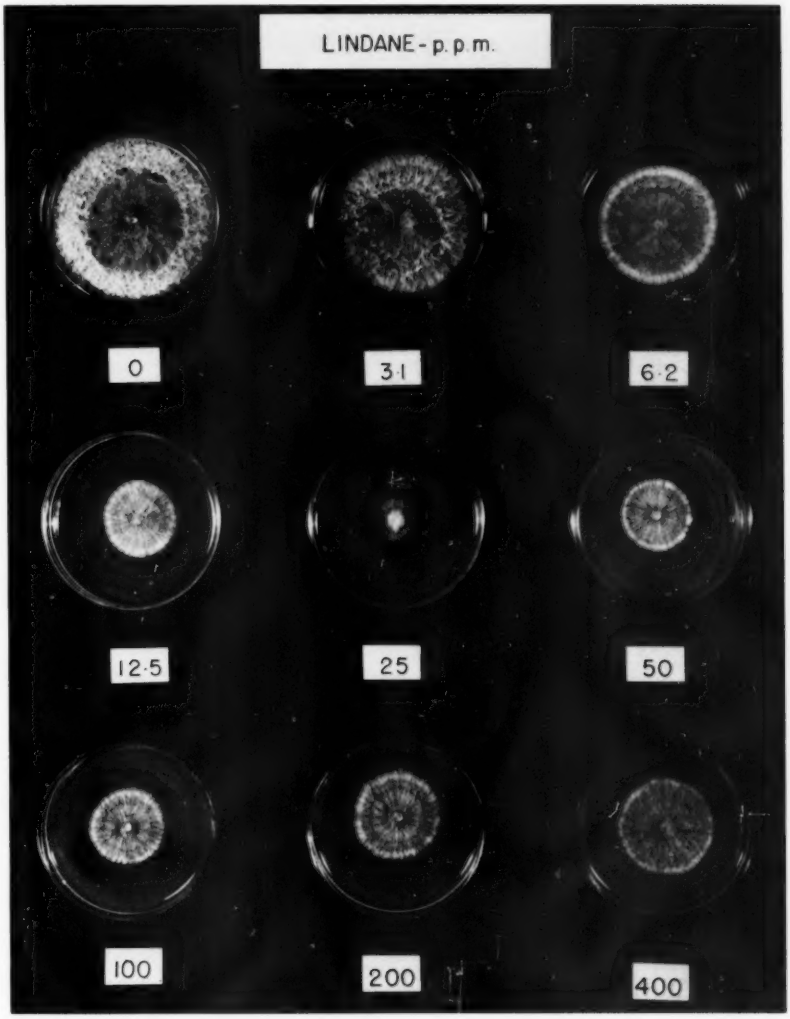


FIG. 2. *Rhizoctonia solani* colonies incubated 3 days on media containing a series of dosages of lindane.

resulted in determinations which were well within the estimated error ($\pm 10\%$) of the over-all measurements. Lindane, chlordane, and heptachlor do not absorb light at wave lengths greater than 220 m$\mu$, the transparent region for TMP. Fortunately, however, the solubility of lindane is great enough to allow analysis by standard methods, and so the polarographic method (6) was applied to the residue remaining after evaporation of the TMP extract. The solubilities of chlordane and heptachlor were not obtained, however, because no sufficiently sensitive method of determination was found.

## Results

Lindane, the first of the insecticides tested against *Rhizoctonia solani*, proved to have marked fungitoxic effects, as indicated by growth inhibition, with an unusual dosage–response relationship. As may be seen from the diameter of the colonies illustrated in Fig. 2, the degree of growth inhibition increased with the dosage up to 25 p.p.m., then decreased to a constant value for all concentrations above 50 p.p.m. Consequently, the dosage–response curve derived from these measurements (Fig. 3) has a regular, fairly steep slope (hereafter referred to as the primary slope) to a sharp peak at 25 p.p.m., followed by a short decline to a horizontal line which did not rise again even at dosages as high as 800 p.p.m. This type of curve was obtained invariably with all lindane samples tested, including commercial, technical, and recrystallized preparations.

Before investigating factors which could account for the inversion in the lindane dosage–response curve, certain tests were made to discount the possibility of its being an artifact introduced through the assay procedure. Although the concentration of acetone employed did retard the growth of the fungus slightly, it did not affect the shape of the curve. This was demonstrated

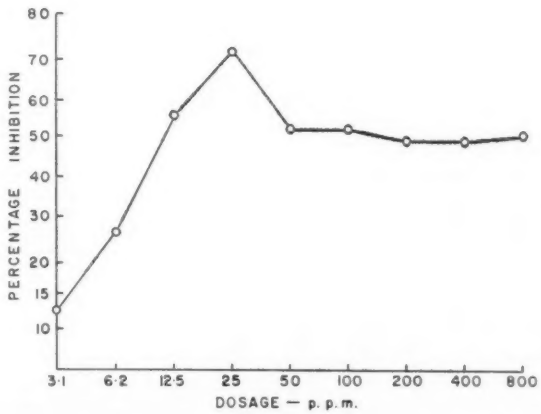


FIG. 3. Dosage–response curve of lindane assayed with *R. solani*.

by substituting suspensions of a commercial wettable powder in 0.5% aqueous solution of methyl cellulose,[3] a dispersing agent, for the stock solutions in acetone. Nor was the shape of the curve influenced by the presence of agar in the medium, as shown by growing the fungus in shake cultures with liquid Czapek's medium and determining inhibition from mycelial dry weights.

Experiments were next conducted to determine whether the factor or factors responsible for the shape of the lindane dosage–response curve are biological, chemical, or physical in nature.

To determine whether or not this type of response was peculiar to the test organism used, sets of plates containing identical lindane dosages were inoculated with *Pythium ultimum*, *Fusarium oxysporum* f. *lycopersici*, and *Monilinia fructicola* in addition to *R. solani*. These four fungi differed widely in their sensitivity to the chemical, but their dosage–response curves were similar in shape with peaks at the identical dosage, as shown in Fig. 4. It appeared unlikely, therefore, that the factor governing the type of response was biological.

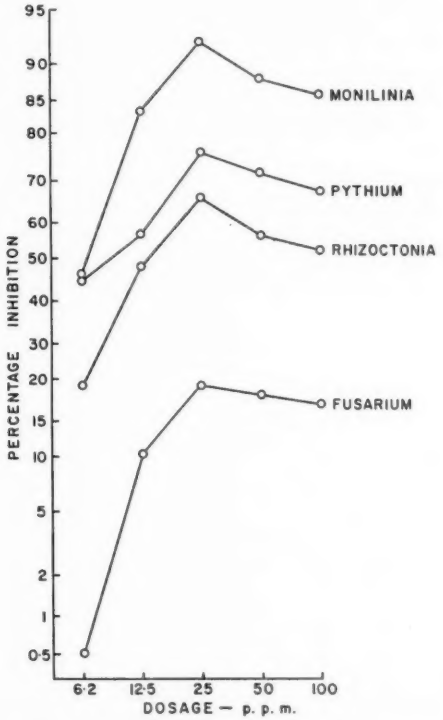


FIG. 4. Dosage–response curves of lindane assayed with various fungi.

[3] "Methocel", Dow Chemical Company.

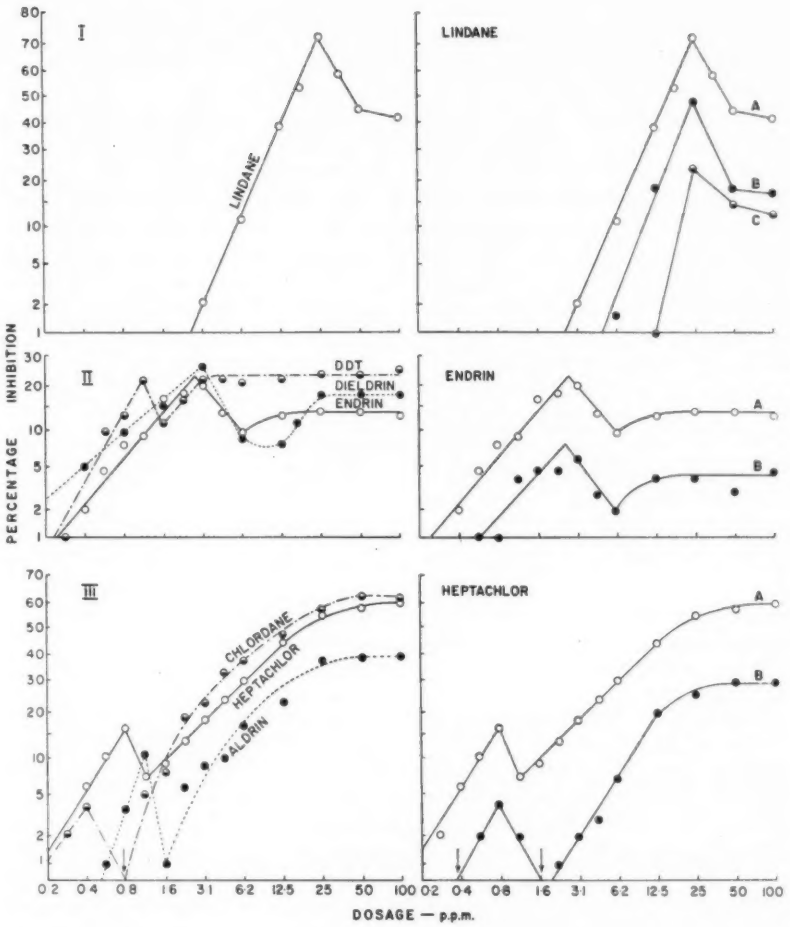


FIG. 5. Dosage–response curves of various insecticides assayed with *R. solani* in two-layered culture plates. Curves on left side grouped according to type of response obtained with toxicant dosages located in upper, inoculated layer of medium. On right side typical curve (A) from each group is shown with corresponding curve (B) obtained with toxicant located in opposite layer. Curve C obtained when lower, treated layer was removed after 24 hours before inoculum introduced. Arrows at base indicate less than 1% inhibition.

The fungitoxicity of other chlorinated insecticides to *R. solani* was next determined in the same way. Their dosage-response curves are shown on the left-hand side of Fig. 5 with a portion of a lindane curve for comparison. The curve for methoxychlor, being almost identical with that for DDT, is omitted. In every case a peak appears at some point on the curve, though of

less magnitude and at a lower dosage than the lindane peak. Since compounds differing in chemical structure produced similar effects, it seemed unlikely that the inversion phenomenon could be explained on the basis of a chemical factor.

The first physical factor considered in relation to fungitoxicity of the insecticides was water solubility. Precise values for the solubility of most of these compounds were not found in the literature, however, and so were determined as outlined under Methods. The results of these determinations are given in Table I, along with a value for DDT obtained from the literature (1). On comparing these data with the dosage–response curves in Fig. 5 a direct relationship between the position and height of the peaks and the water solubility of the compounds becomes apparent. Lindane, the most soluble of the group, has the highest peak appearing at the highest dosage.

TABLE I

Solubilities of some chlorinated insecticides in water, in mg/l. (p.p.m.)

| | Temperature | | |
|---|---|---|---|
| Insecticide | 25° C | 35° C | 45° C |
| Lindane | 7.3 | 12.0 | 14.0 |
| Dieldrin | 0.25 | 0.54 | 1.0 |
| Endrin | 0.23 | 0.38 | 0.51 |
| Aldrin | 0.20 | 0.39 | 0.79 |
| Methoxychlor | 0.10 | 0.20 | 0.40 |
| DDT | 0.037* | — | — |

*Value from literature (1).

If the degree of inhibition of the test organism is determined by the amount of toxicant in solution in the medium, however, we should expect the dosage–response curve to rise with increasing dosages up to the saturation point, then level off. Actually, the lindane curve (Fig. 3) fits this description if we ignore the peak for the moment and extend the horizontal line back to intersect the slope. The dosage at the point of intersection is in the vicinity of the determined solubility for lindane at 25° C. To account for the additional inhibition in the peak area in terms of water solubility then, it is necessary to postulate that supersaturation occurs within this dosage range, then normal saturation again at all higher dosages.

Evidence in support of this hypothesis was found on close examination of the culture plates containing the range of lindane dosages. In freshly poured plates fine crystals of lindane could be seen in the agar where 50 p.p.m. or more of lindane had been introduced, but none at 25 p.p.m. or less even with the microscope. After 3 or 4 days, however, aggregations of coarse crystals, such as may be seen in the center plate of Fig. 2, or extensive "snowflake" patterns of finer crystals could be seen, with the unaided eye, only in plates containing 25 p.p.m., whether inoculated or not. These crystals also appeared at about the same time in liquid media to which 25 p.p.m. pure lindane dissolved in acetone, or wettable powder suspended in methyl cellulose, had

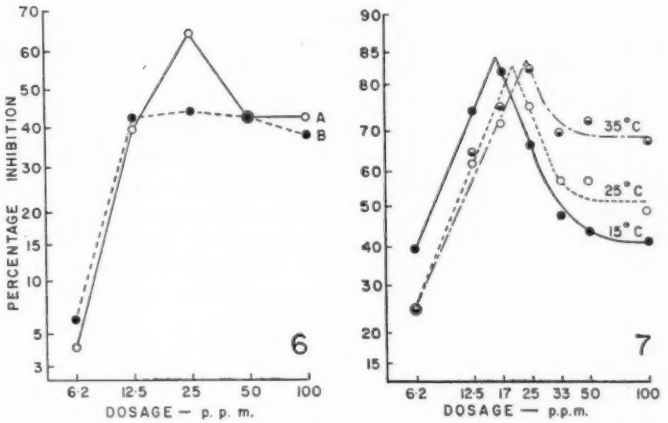


FIG. 6. Lindane dosage–response curves derived from plate cultures inoculated with *R. solani* (A) before and (B) after crystals formed at 25 p.p.m. dosage.
FIG. 7. Lindane dosage–response curves derived when *R. solani* plate cultures were incubated at various temperatures.

been added. When inoculation of a series of lindane-treated plates was delayed 4 days, until after these crystals had formed, a dosage–response curve with no peak was obtained (B, Fig. 6).

If inhibition of mycelial growth is related to the concentration of toxicant in solution, we would expect the dosage–response to be affected by temperature. The effect of temperature on the inhibition of *R. solani* by lindane was determined by incubating identical sets of plates at 15°, 25°, and 35° C. Colony diameters were measured after 4, 3, and 2 days respectively because of the differences in growth rates. From the dosage–response curves shown in Fig. 7, it is apparent that both the position of the peaks and the levels of the plateaus varied with the temperature. Again, delayed crystal formation was observed at the dosage at which the peak occurred in each case.

Although supersaturation could account for the peak on the dosage–response curve of each insecticide tested, the response at dosages above this point for some of the compounds suggested that an additional physical factor was involved in these cases. The curves illustrated in Fig. 5 are grouped according to the type of response in this dosage range. The lindane curve is unique in that it maintains a constant level following the decline from the peak (see also Fig. 3). The curves for DDT, dieldrin, and endrin (group II) have a slight second slope to a plateau which does not exceed the peak in height. Methoxychlor also belongs in this group. The curves for chlordane, heptachlor, and aldrin (group III) are distinctly bimodal, having a long second slope ending at a level higher than the peak.

The possibility that mycelial growth could be affected by the vapor of an insecticide in addition to the aqueous solution was next investigated. As a preliminary test colonies of *R. solani* were incubated on untreated medium

TABLE II

Inhibition of growth of *R. solani* colonies exposed to vapors from pure crystals of various insecticides in relation to the vapor pressures of these compounds

| Insecticide | Group* | Vapor pressure,† mm Hg at 25° C | Inhibition,‡ % |
|---|---|---|---|
| Heptachlor | III | $3\times10^{-4}$ | 36.0 |
| Chlordane | III | $1\times10^{-5}$ | 32.3 |
| Lindane | I | $9.4\times10^{-6}$ | 34.3 |
| Aldrin | III | $6\times10^{-6}$ | 22.0 |
| Endrin | II | $2\times10^{-7}$ | 7.0 |
| DDT | II | $1.9\times10^{-7}$ | 0 |
| Dieldrin | II | $1.8\times10^{-7}$ | 4.5 |
| Methoxychlor | II | ? | 4.0 |

*See Fig. 5 and text.
†Values as reported in literature.
‡Mean values from several determinations.

over a layer of fine crystals of each chemical deposited on the lids of the dishes following evaporation of an acetone solution. The percentage inhibition caused by each insecticide tested is shown in Table II with the vapor pressure of the compound as reported in the literature. It should be noted that insecticides of groups I and III caused much greater inhibition than did those of group II, which have very low vapor pressure.

Further evidence of the vapor effect on mycelial growth was found in another test. Inoculated plates of chlordane-treated medium were incubated without their glass lids, being inverted on wire racks covered with cheesecloth. When the rate of dissipation of chlordane vapor was increased in this way, 10 times the dosage was required to produce an effect equivalent to that obtained in dishes closed by the glass covers.

The vapor effect was investigated still further by exposing the test organism to a gradient of vapor concentrations. This was achieved through the use of plates containing two layers of medium separated by an air space (Fig. 1). Parallel sets of these plates were used, one having the dosage series of each chemical in the upper, inoculated layer and the other having the same dosages in the opposite layer. On the right-hand side of Fig. 5 a typical curve (A) from each group is redrawn with the curve (B) obtained when the same dosages were applied in the layer of medium below the inoculum. Since the curves A and B are parallel in each case, it may be concluded that the magnitude of the second slope of the dosage–response curve for each compound is determined by the concentration of vapor to which the organism is exposed. The upper limit of the slope is determined by the vapor pressure of the compound.

This technique does not permit complete separation of the toxic effect of a compound as a vapor from its effect in solution because it is possible for the vapor from the lower layer of medium to dissolve in the untreated layer. This was demonstrated by leaving the plates closed for 24 hours after pouring, then removing the lower treated layer before inoculating the upper layer. The third response curve (C) for lindane, which has relatively high vapor

pressure and solubility, was obtained in this way. This effect could not be detected with any of the less soluble insecticides regardless of their vapor pressure.

## Discussion

Certain insecticides when applied to the soil can cause a reduction in the population of soil-borne fungi, according to numerous reports in the literature (4). However, attempts to demonstrate fungitoxicity by sowing spores of various fungi on culture media treated with these compounds have invariably given negative results (8, 9, 12, 13). Inhibition of mycelial growth, on the other hand, has been demonstrated by inoculating treated media with mycelium of fungi. Crude BHC was shown in this way to be toxic to *Rhizoctonia solani* (10). Differences in antifugal activity between four of the isomers of BHC have also been demonstrated. The delta isomer causes marked inhibition of several fungi (2, 5, 13). The gamma isomer is less active and more selective, inhibiting *Diplodia zeae* but not *Fusarium oxysporum* (5). The alpha and beta isomers are inactive (2, 5).

The plate culture assay method employed in the present study made it possible not only to demonstrate fungitoxicity with each of the insecticides tested, but also to measure quantitative differences in mycelial growth so that dosage–response relationships could be established. Although none of the insecticides proved to be fungicidal, the observed responses to sublethal dosages are of fundamental interest in the study of modes of action in relation to physical factors. The bimodality of the dosage–response curves obtained can be fully explained on the basis of two physical properties of the compounds, water solubility and vapor pressure.

The relationship between water solubility and inhibition of mycelial growth is shown by the first part of each dosage–response curve. The initial rise in each case is a normal linear curve, indicating that the degree of inhibition varies with the amount of toxicant in solution in the medium. The magnitude of this initial rise is limited by the inherent solubility of the compound in water. The effectiveness of the various insecticides tested may be expressed as $ED_{50}$ values which might be obtained if there were no solubility restrictions. If these values are taken as the concentration corresponding to the point of intersection of the extrapolated initial slope and the 50% inhibition line, they prove to be of the same order for all of the compounds, ranging from 3 to 15 p.p.m.

The peak on each dosage–response curve is apparently due to a state of temporary supersaturation which occurred under the conditions of these experiments. When introduced into the medium the compounds were dissolved either in acetone or in the micellar phase of the dispersing agent. Dilution of these initial solutions by the medium resulted in a reduction of solubility so that when the final concentration of a given compound exceeded its solubility in the medium the excess solute could be removed only by the formation of crystals. However, such crystals cannot form unless provided with nuclei.

According to the accepted concepts of crystallization (11), these nuclei arise spontaneously in a supersatured solution as the result of chance formation of aggregates of molecules which, if they exceed a certain size, become stable crystals to which other molecules can add. The formation of nuclei may be added by the presence of small solid particles, such as air-borne dust, but in general the probability of their formation is dependent on the degree of supersaturation. Once nuclei have formed, the growth of the crystals, and hence the drop in supersaturation, is rapid.

In the case of lindane, for example, the time required for crystal formation at dosages of 25 p.p.m. or less was longer than the 3-day growth period. Consequently, the organism was subjected over the entire test period to solutions with concentrations equivalent to the dosage applied. At higher dosages, however, crystal formation became more rapid, so that at 50 p.p.m. and higher crystals appeared soon after the plates were poured and the organism was exposed to a normally saturated solution only. As a result, inhibition reached a peak at 25 p.p.m. and then declined to a normal value at dosages of 50 p.p.m. and greater.

Inhibition of mycelial growth by the vapors from various insecticides is indicated by the latter part of each dosage–response curve. The pressures which these vapors reached within the closed (but not sealed) Petri dishes were determined by two factors: the rate of evaporation from solutions or crystal surfaces and the rate of diffusion of this vapor out of the dish into the surrounding atmosphere. Loss by diffusion was constant but the rate of evaporation was dependent on the concentration of the solution in the medium and the surface area of solid exposed at or near the agar–air interface. Thus at low dosages the degree of inhibition varied with the dosage and a peak also appeared on the vapor curve where supersaturation of the medium occurred. With increasing dosages above this point increasing numbers of crystals formed, providing greater surface areas until finally the rate of evaporation exceeded diffusion to such a degree that saturation of the gas phase was virtually achieved. It should be noted that the maximum degree of inhibition shown on each vapor curve (B) of Fig. 5 is of the same order as the value listed in Table II for inhibition induced by pure crystals of each compound deposited on the glass lids. The second rise in the dosage–response curve appears, therefore, to be due to the effect of the increasing vapor concentration on the exposed aerial mycelium. This interpretation is supported by the experiment in which the rate of diffusion was increased by removing the glass covers from the culture dishes. Eliminating vapor loss completely by sealing the dishes proved impractical since the consequent exclusion of oxygen interfered with the normal growth of the organism.

The insecticides arbitrarily grouped together according to type of dosage–response are similar with respect to the two physical properties considered. Lindane, the only compound having a curve with a major primary slope, is in a class by itself with respect to solubility. There is no second rise on its curve, however, despite its relatively high vapor pressure and the demonstration that lindane can move across an air space between two layers of agar.

This does not necessarily mean that the vapor of lindane is not toxic, but rather that its vapor effect is masked by its solution effect whereby the maximum inhibition by this compound is already achieved before the vapor attains toxic concentration. Heptachlor, chlordane, and aldrin (group III) have distinctly bimodal curves with minor primary and major secondary slopes. These compounds are characterized by very low water solubility and relatively high vapor pressure. The latter property largely accounts for the strong fungitoxicity of this group. The curves for DDT, methoxychlor, dieldrin, and endrin have only minor primary and secondary slopes. These compounds are weakly fungitoxic because both water solubility and vapor pressure are low.

It was demonstrated with lindane that the proportions of the dosage-response curve can be modified by varying the temperature at which the cultures are incubated. This is largely a physical effect since temperature affects both solubility and rate of crystal formation. That temperature may have a biological effect as well is suggested by the fact that the primary slopes in Fig. 7 do not coincide. The sensitivity of the organism may vary with its growth rate which is affected by temperature.

Another aspect of the problem is brought out by a comparison of the relative effectiveness of these compounds as vapors and in solution. As we have seen, saturation of the vapor phase results in a maximum percentage inhibition by compounds in groups II and III. These maximum values, obtained from the curves in Fig. 5, are given in the second column of Table III. The concentration of each compound producing this maximum effect (i.e. its concentration in the saturated vapor phase) has been calculated from its vapor pressure and recorded in column 3 of the table. The concentration of each insecticide in the aqueous phase which would be required to produce the same degree of inhibition as the saturated vapor is also shown in column 4. This value was obtained from the appropriate curve in Fig. 5 by reading off the initial linear portion (or extrapolation thereof) against the corresponding value of column 2. Finally, the ratio of these concentrations (i.e. the ratio of the concentration in the liquid phase to that in the vapor phase producing an equivalent effect) is given in the last column of Table III. It is interesting to note that for most

TABLE III

A comparison of the fungitoxic effect of vapors of various insecticides with their effect in solution

| Insecticide | Maximum inhibition,* % | Vapor concentration,† moles/l. | Solution concentration,* moles/l. | Ratio solution/vapor |
|---|---|---|---|---|
| Endrin | 14 | $1\times10^{-11}$ | $4\times10^{-6}$ | $4\times10^{5}$ |
| Dieldrin | 18 | $1\times10^{-11}$ | $5\times10^{-6}$ | $5\times10^{5}$ |
| DDT | 23 | $1\times10^{-11}$ | $3\times10^{-6}$ | $3\times10^{5}$ |
| Aldrin | 39 | $1\times10^{-10}$ | $6\times10^{-6}$ | $6\times10^{4}$ |
| Heptachlor | 60 | $2\times10^{-8}$ | $9\times10^{-6}$ | $5\times10^{2}$ |
| Chlordane | 63 | $6\times10^{-10}$ | $19\times10^{-6}$ | $3\times10^{5}$ |

*Values derived from curves in Fig. 5 (see text).
†Calculated from vapor pressure.

of these compounds this ratio is of the order of $10^6$, which indicates that a molecule in the vapor phase is $10^5$ times as effective as one in solution. This is rather surprising since the ultimate effect of these compounds would be expected to take place in the aqueous phase (i.e. within the organism), and it should not matter by what route the compound reaches this phase. The apparent difference in effectiveness between the solution and the gas phase might be explained by a possible difference in permeability between aerial and submerged mycelium.

Anomalous relationships between insecticide dosage and fungitoxicity have been reported by other workers but not interpreted in terms of physical factors. Block (2), evidently using a resistant test organism, found gamma BHC by itself to be relatively inactive even at high dosages. In mixtures containing a constant concentration of delta BHC and varied concentrations of gamma isomer, however, he found that fungal inhibition increased with the gamma dosage until the quantity of the two isomers was equal, then decreased with further increase in gamma isomer. He concluded that the gamma isomer acts synergistically to increase the activity of the delta isomer. It is possible, however, that the effect of the gamma is additive rather than synergistic, with maximum activity occurring at the point of supersaturation of the medium as demonstrated in the present study. Gar *et al.* (5) also observed maximum inhibition of fungal growth at a given concentration of both delta and gamma isomers of BHC. Their explanation of the mode of action on the basis of a chelation mechanism is not supported by experimental evidence, however.

The phenomenon of bimodality in dosage–response, first observed with thiram by Dimond *et al.* (3), has been encountered frequently with organic fungicides and interpreted in various ways. The possible role of physical factors, particularly supersaturation, should not be overlooked in considering mechanisms responsible for apparent anomalies in fungicidal action.

Apart from the theoretical considerations discussed here, fungitoxic effects of insecticides may have practical significance in respect to seed and soil treatments. In either a dissolved or a vapor state in a more or less closed system the compounds could move within the soil from the point of application and, by retarding the growth of soil-borne pathogenic fungi, provide a measure of protection against seedling diseases during the brief susceptible stage in the development of a host plant.

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# CAMBIAL BEHAVIOR WITH REFERENCE TO CELL LENGTH AND RING WIDTH IN THUJA OCCIDENTALIS L.[1]

M. W. BANNAN

## Abstract

The relationship between ring width and length of wood cells was studied by reference to mature white cedar trees of various growth patterns. These included trees of similar diameter but diverse growth rates, trees with transition from wide to narrow rings or from narrow to wide rings in their peripheral growth, and trees with rings varying in width in different radii. Although much fluctuation occurred among individuals there was in general an inverse relationship between cell length and ring width. On the other hand, the variations in frequency of pseudotransverse divisions in the cambium, which might be expected to have a profound influence on cell length because of their involvement in cambial cell multiplication, were not obviously related to the growth rate. The frequency of pseudotransverse divisions in the fusiform initials apparently is geared neither to circumferential expansion nor to number of periclinal divisions in the cambium but rather seems to be related simply to linear radial increment.

## Introduction

The size of the cellular components of wood has been the subject of investigation for many decades. Much attention has been given to such aspects as the influence of environmental factors on cell size and wood texture, trends of variation in different parts of the tree, and genetic control. Since the length of wood cells in conifers is closely related to the size of the originating cambial cells, it might be expected that events in the production and perpetuation of the latter would have an important bearing on wood characteristics. The present paper deals with some aspects of this problem, particularly with the interrelations between rate of growth, cell length, and frequency of multiplicative divisions in the cambium.

## Ring Width and Cell Length

Several reports have been published on the relationship between tracheid length and ring width in conifers. In most of the earlier studies the comparison was based on rings of similar age. Adams (1), Berkley (8), Kienholz (13), Lee and Smith (15), MacMillan (17), and Mell (18) observed longer cells in any given ring in the more actively growing trees. In a review article Spurr and Hyvärinen (19) concluded from the literature that rapid growth leads to long fibers, or is related to long fibers, and stated that the question of the characteristic that should be taken as the measure of growth remains unanswered. Other investigators, however, have reported that among trees of similar diameter the tracheids tend to be longer in the slower growing specimens. For instance, Chalk (10) noted in *Picea sitchensis* that in any given

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ring tracheid length was greater on the side of the stem where the width was less. Helander (12) deduced from his own observations and the work of certain contemporaries that "in corresponding parts of trees with narrow annual rings the tracheids are longer than those in trees with broad annual rings". From the pith outward in *Larix* spp. Liang (16) found that as ring width decreased from one period to another the average tracheid length tended to increase. In *Pinus radiata* Bisset, Dadswell, and Wardrop (9) discovered that in general wide growth rings corresponded with a short tracheid length and narrow growth rings with a long tracheid length. Echols (11) was unable to find a direct relationship between tracheid length and ring width in slash pine, and concluded that tracheid length was under rigid genetic control.

In extensive studies of various phases of cambial activity in *Thuja occidentalis* the author found, when comparing trees of similar diameter, that the specimens with the narrower rings tended to have longer wood cells (3). Continued observations have confirmed the earlier studies. The results obtained from examination of the peripheral growth of many specimens, divided into three categories of stem size, are assembled in Table I. The data show the mean length of cells at the time of cambial cell multiplication, that is, when the fusiform initials underwent pseudotransverse division. Similar trends are apparent in all three categories of stem size. Increase in ring width is accompanied by a decrease in cell length at the time of anticlinal division. Much variation occurs from tree to tree, as shown in Fig. 1, but nonetheless a definite trend is evident. Statistical analysis of 145 trees in the size range of 2.5–3.0 dm diameter reveals a correlation coefficient of $-0.51$ with a standard error of 0.06. Since the correlation coefficient is more than eight times its standard error the coefficient is significantly different from zero.

TABLE I

Ring width, frequency of pseudotransverse division, and cell length (at pseudotransverse division) in the peripheral growth of white cedar stems 1.5–5 dm in diameter

| Diameter of bole, dm | No. of trees | Ring width, mm | Frequency of pseudo-transverse divisions per cm of radial increment | Mean cell length at pseudo-transverse division, mm | Percentage survival of fusiform initials |
|---|---|---|---|---|---|
| 1.5–2 | 4 | To 1.0 mm | 1.9 | 2.91 | 10 |
| | 10 | 1.01–2.00 | 2.4 | 2.67 | 12 |
| | 3 | >2.00 | 1.9 | 2.64 | 24 |
| 2.5–3 | 7 | 0.10–0.30 | 3.0 | 3.10 | 9 |
| | 29 | 0.31–0.50 | 1.9 | 3.05 | 4 |
| | 42 | 0.51–0.75 | 2.0 | 3.00 | 3 |
| | 37 | 0.76–1.00 | 2.1 | 3.02 | 6 |
| | 45 | 1.01–1.50 | 2.2 | 2.87 | 8 |
| | 20 | 1.51–2.00 | 2.3 | 2.83 | 13 |
| | 14 | 2.01–3.00 | 2.0 | 2.78 | 18 |
| | 16 | >3.00 | 2.0 | 2.57 | 20 |
| 4–5 | 12 | To 1.0 mm | 2.7 | 2.95 | 8 |
| | 13 | 1.01–2.00 | 2.5 | 2.88 | 10 |
| | 9 | >2.00 | 2.6 | 2.47 | 11 |

As mentioned earlier the data in Table I and Fig. 1 were obtained by sampling different trees. In order to eliminate the genetic factor as a variant additional observations were made on trees which showed marked fluctuations in their growth rates. Trees were sought which fell into two classes, one in which there was a marked contraction in width of the peripheral rings, and a second class in which a decided acceleration of growth occurred during the development of the final rings. In 24 trees belonging to the first category the ring width was reduced from a mean of 1.48 mm to a mean of 0.66 mm in the outermost rings. The frequency of pseudotransverse division dropped from 2.3 per cm of radial increment to 1.8 per cm, and mean cell length at the time of pseudotransverse division increased from 2.85 mm in the penultimate rings to 3.00 mm in the final rings. Mean length of all tracheids followed suit, rising from 2.58 mm to 2.80 mm. Analysis of individual trees showed that 17 of the 24 conformed to the trend, 3 exhibited no change with declining vigor, and 4 were contrary.

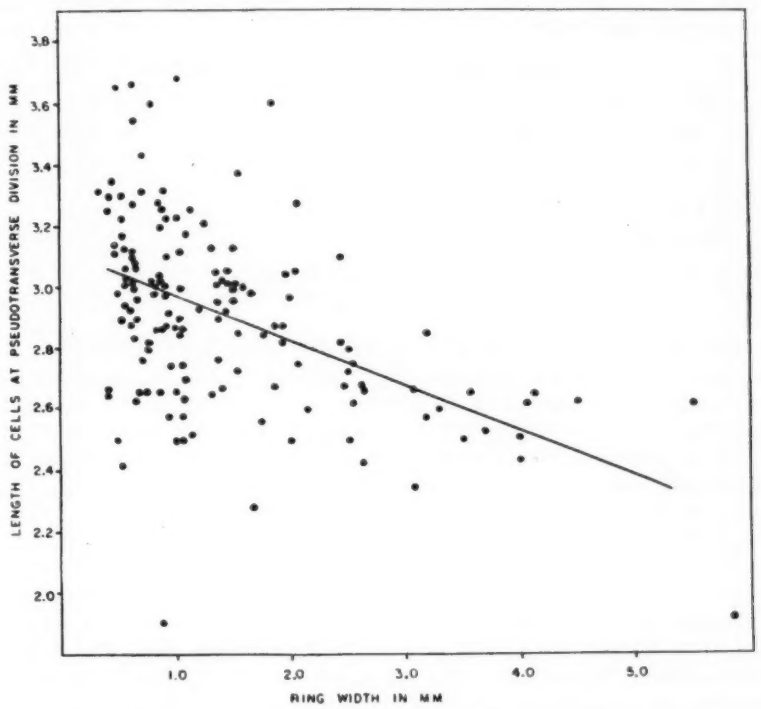


FIG. 1. Relation between cell length at the time of pseudotransverse division of the fusiform initials in the cambium and ring width as determined from the peripheral growth of white cedar trees 2.5–3.0 dm in diameter.

Trees in the second category, distinguished by an appreciable widening of the rings in the final growth, were more difficult to find. In the 16 which were studied the ring width increased from an over-all average of 1.03 mm in the penultimate rings to 2.11 mm in the outermost rings. Cell length at pseudotransverse division decreased slightly from a mean of 2.77 mm to 2.68 mm in the final rings. Among the 16 trees 11 followed the trend, 1 was without inclination, and 4 were contrary.

Finally, a study was made of cell length and frequency of anticlinal division in trees which exhibited fluctuations in ring width in different sectors. Here the investigations were confined to boles of rounded contour without decided convexities or deep grooves, for it has been shown (6) that in such stems cambial behavior is profoundly altered in the grooves. In the 10 specimens which were examined the width of the eccentric rings ranged from a mean maximum of 2.53 mm to a mean minimum of 0.74 mm. The over-all average cell length at pseudotransverse division was 2.51 mm in the wide portions of the rings and 2.67 mm in the narrow parts. Thus in all these comparisons a similar trend is evident. Increase in ring width is accompanied by a slight reduction in cell length.

## Frequency of Pseudotransverse Divisions

In the cambium of conifers accommodation to increasing girth is accomplished mostly by increase in number of fusiform initials. The addition is achieved by pseudotransverse division which involves the formation of a radially oriented, more or less sigmoidal, wall extending across the center of the dividing cell (2, 14). The two newly formed daughter initials are each somewhat more than one-half the length of the original cell. Thereafter cell length increases by tip growth and when full size is attained another pseudotransverse division occurs, thus continuing the repetitive cycle of cell multiplication and enlargement.

Since the xylem cells elongate only slightly during differentiation and maturation, their length is determined largely by that of the cambial cells from which they are derived. A seemingly reasonable deduction would be that length of the latter is in turn related to events in their production, such as the frequency of multiplicative division and the rate of cell elongation between successive divisions. In other words, it might be expected that variations in length of wood cells are related to the mechanism of cambial cell growth.

Calculations of the frequency of pseudotransverse divisions were made by the method described earlier (6). As shown in Table I successive pseudotransverse divisions of fusiform initials occurred at a mean rate of slightly more than two for every centimeter of xylem increment. Wide fluctuations were noted in different trees, with a range from 0.6 to more than 10 successive divisions per centimeter of xylem increment, but the over-all mean was similar in both slow-growing and vigorous trees. The results indicate that the frequency of multiplicative divisions in the cambium is independent of the growth rate.

It has been pointed out that the production of new fusiform initials in the cambium by pseudotransverse division far exceeds the number required to accommodate the cambium to increasing girth (3, 20). Wastage is high, and sometimes the loss of initials by reduction to potential ray initials or maturation to permanent elements almost equals the new production. Although the frequency of multiplicative division varies widely in different samplings, and the ratio of survival fluctuates accordingly, some trends were noted in the trees examined. As shown in Table I the ratio of survival of fusiform initials was highest in the trees of most active growth. When the ring width exceeded 3 mm the survival rate was 20%. In other words approximately one out of every five fusiform initials newly formed in pseudotransverse division continued to function for an indefinite period. As the growth rate slackened the survival rate dropped until in those trees with the outermost rings 0.4–0.7 mm wide the loss of initials almost equalled the new production. Accommodation to the slowly increasing girth was apparently achieved by increase in cell length. In stems with narrow rings, 0.1–0.4 mm wide, there was a small net gain. It may also be noted in Table I that the percentage survival of fusiform initials after multiplicative division was slightly higher in the peripheral growth of the smaller (and presumably younger) trees than in the larger trees.

The shorter length of the cells in wider rings is not due to an increase in the frequency of pseudotransverse division. As shown in Table I the over-all rate of anticlinal division is similar, with minor fluctuation, in trees of different growth rates. This relatively uniform pace might be interpreted as indicating that the rate of multiplicative division among the fusiform initials is geared to circumferential expansion, but such a conclusion is unjustified. In Table II data are provided on cell length and frequency of anticlinal division in the peripheral growth of trees approximately 1.5, 2.5, 3.0, and 4.0 dm in diameter.

TABLE II

Frequency of pseudotransverse division and cell length in the peripheral growth of white cedar stems of similar growth rates but differing in size

| Diam. of bole, dm | No. of trees | Mean ring width, mm | Frequency of pseudotransverse divisions per cm of radial increment | Mean cell length at pseudotransverse division, mm |
|---|---|---|---|---|
| 1.5–2 | 16 | 1.44 | 2.3 | 2.68 |
| 2.5 | 54 | 1.42 | 2.1 | 2.87 |
| 3.0 | 35 | 1.42 | 2.3 | 2.90 |
| 4–5 | 23 | 1.43 | 2.5 | 2.84 |

Mean frequency of pseudotransverse division varied only slightly in the different size categories, and was actually somewhat higher in old as compared with young trees. Most trees were not perfectly round, but checks on stems with marked eccentricities failed to reveal any relationship between the frequency of pseudotransverse division and the radius of curvature in the sectors studied (6). Examination of some very old conifers yielded astonishing results. For instance, in the final growth of trees of *Sequoia sempervirens* and *S. gigantea* which were 2–6 *meters* in diameter, the rate of anticlinal division

ranged up to as much as five successive divisions per centimeter of radial increment. It is clear from these observations that the rate of anticlinal division in the cambium is not geared to circumferential expansion.

The fact that pseudotransverse divisions occur at a relatively high rate in old stems where circumferential expansion is minimal might be regarded as indicating that the relationship is with radial increment as expressed by number of periclinal divisions in the cambium. However, in this connection certain aspects of cambial growth should be recalled. Within the cambial zone are found the uniseriate layer of initiating cells and the derived xylem mother cells. In actively growing trees the latter comprise a zone several cells wide and successive divisions occur before maturation. Thus for every periclinal division of the fusiform initial several xylem cells accrue (5). By way of contrast, in slow-growing trees the zone of xylem mother cells is narrow and fewer divisions occur before maturation. Hence the fusiform initials themselves must divide more frequently to produce a given number of xylem cells. If the number of periclinal divisions in the fusiform initials were the principal factor determining the spacing of pseudotransverse divisions, the frequency of the latter should be lower in vigorous than in slow-growing trees, because the number of times that the fusiform initials divide periclinally is less per centimeter of xylem increment in vigorous than in slow-growing trees. In point of fact no consistent differences in rate of anticlinal division were revealed in trees with annual increments over the size range of 0.3–5 mm. Only in occasional very slow growing stems, with rings 0.1–0.2 mm wide, was a marked increase in frequency of pseudotransverse division noted.

Apparently fusiform initials undergo pseudotransverse divisions at periodic intervals, the spacing of which is not related to circumferential expansion or number of periclinal divisions in the initials. Actually the closest relationship appears to be with linear radial distance. In trees exceeding 1.5 dm in diameter the rate of pseudotransverse division was found to average 2–3 per cm of xylem increment regardless of tree size or rate of growth.

It is also manifest that the length of fusiform initials is not influenced by the rate of pseudotransverse division to the extent that might be expected.

TABLE III

Relation between rate of pseudotransverse division in the cambium and cell length in the peripheral growth of white cedar stems 2.5–3 dm in diameter

| | | Trees with frequency of pseudotransverse division below average | | Trees with frequency of pseudotransverse division above average | |
|---|---|---|---|---|---|
| Ring width, mm | No. of trees | Freq. ptr. div. per cm of increment | Mean cell length at ptr. div., mm | Freq. ptr. div. per cm of increment | Mean cell length at ptr. div., mm |
| 0.31–0.50 | 29 | 1.1 | 3.12 | 4.0 | 2.89 |
| 0.51–0.75 | 40 | 1.1 | 3.12 | 3.1 | 2.78 |
| 0.76–1.00 | 32 | 1.2 | 3.15 | 3.1 | 2.92 |
| 1.01–1.50 | 44 | 1.4 | 3.03 | 3.2 | 2.72 |
| 1.51–2.00 | 23 | 1.7 | 2.97 | 3.4 | 2.67 |
| >2.00 | 27 | 1.3 | 2.73 | 2.9 | 2.46 |
| Mean | | 1.3 | 3.02 | 3.3 | 2.74 |

Although a sharply increased rate of multiplicative division in the cambium does produce a reduction in mean cell length, the shortening is by no means proportional to the increase in rate of division. As shown in Table III a two and one-half fold increase in the frequency of pseudotransverse division resulted in an over-all reduction in cell length of only 10%.

The surprisingly limited effect of high rates of pseudotransverse division on cell length is due in large measure to other factors which operate to prevent drastic reductions in mean length of the cells. As a rule accelerated multiplicative divisions in the cambium are accompanied by faster rates of cell elongation between divisions (in terms of radial increment) so that the newly formed short cambial cells soon become longer. Further, the shortest of the fusiform initials arising in pseudotransverse division are usually eliminated in the intracambial competition between cells and only the longest survive (7). As the frequency of division rises this competition intensifies so that the factor of selection operating to bring about survival of the longest cells becomes increasingly more important.

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# THE VEGETATION OF NORTHERN MANITOBA

## IV. THE CARIBOU LAKE REGION[1]

J. C. RITCHIE


## Abstract

Based on the findings of one season of field work, an account is given of the vegetation and flora (vascular plants) of the immediate vicinity of Caribou Lake in northeast Manitoba. The plant communities are grouped according to physiographic position. Their chorology is presented in the form of a map which was compiled from interpretation of vertical aerial photographs of the area. The appearance of the various vegetation types on the aerial photographs is described.

The prevalent vegetation of mesic sites is a tundra community dominated by ericoid shrubs. There is strong evidence that much of this treeless vegetation occupies sites where trees once grew; they have been removed by fires. Recent alluvium bears stands of *Picea glauca* (white spruce) which shows good growth. Shallow wet peats are covered by black spruce stands with shrubs and mosses, chiefly *Sphagnum*. Deeper peats, usually with shallow active layers, bear heath or sedge–cottongrass tundra.


## Introduction

The specific aims of this paper are to outline certain of the features of the vegetation and flora of a forest–tundra region of Manitoba, to evaluate the status of the vegetation, to indicate any association of vegetation type with physiography, and to point out those diagnostic features by which vegetation types can be recognized on vertical aerial photographs of the area. An accident during the field work deprived the author of certain items necessary for the preparation of a detailed report; accordingly, the emphasis here is on the spatial arrangements of the major communities of the region and the use of aerial photographs in depicting their areal relationships on map, rather than the equally desirable compilation of the detailed floristic and structural aspects of the vegetation. This account is designed as one step towards the preliminary objective of the general investigation, which is to accumulate accurate geographical and botanical reports of the major vegetation zones of Manitoba which lie north of the 56th line of latitude, an area which includes two main geographical regions, the Pre-Cambrian shield and the southern or continental portion of the Hudson Bay Lowlands.

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Contribution from the Department of Botany, University of Manitoba, Winnipeg, Manitoba.

## Climate and Physiography

It is possible to provide only a general summary of the climate of the area, since the nearest recording meteorological station is 140 km distant, at Churchill. The following comments have been abstracted from the publications of Saunderson (10), Thomas (12), and Kendrew and Currie (4). The summers are cool and dry and the winters very cold with strong north, northwest, and northeast winds. The summer is short, lasting for only about 60 days, from the end of June until the end of August. Only the months between and including June and September have mean temperatures above 0° C. There is a relatively high summer concentration of thermal efficiency (type $c'_1$ of Saunderson (10)), and slight moisture deficiency. The area lies within Saunderson's (10) moist subhumid (C2) region, and has an average annual potential evapotranspiration of about 32 cm; that is, within the microthermal climatic type.

Throughout the area relief is generally low, the main landform being undulating till deposits which are replaced eastwards by extensive, flat deposits of peat overlying clays. Local relief is provided by coarse, usually sandy deposits—ice-contact drift which has been reworked to greater or lesser extents by wave action during postglacial periods of inundation. Approximately 15 km due south of Caribou Lake a large fragment of esker ridge (? kame) was examined; its slopes are steep (35–40°) and a number of readings with an aneroid barometer indicated that the highest point was approximately 90 m above the level of the surrounding lowlands.

Several eskers occur in the area, consisting of sinuous ridges of coarse sands and gravels, usually lying in a north–south direction and often with large surface boulders. Several show evidence of having been modified by wave action. Beach ridges and spits are common in the immediate vicinity of Caribou Lake.

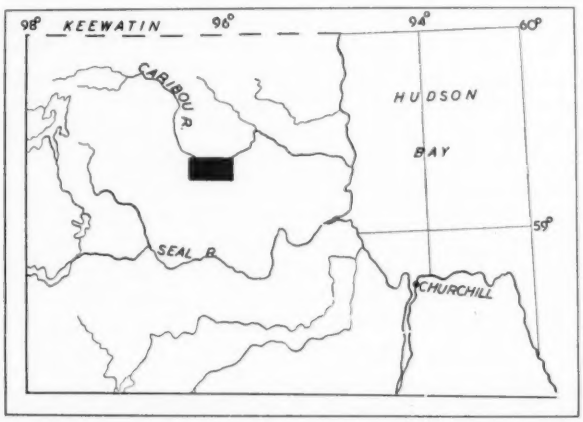


FIG. 1. A map of the northeast corner of Manitoba showing the geographical position of the area of study (shaded rectangle). This is the area mapped in Fig. 3 of this paper.

A number of discrete areas of orientated ridges occur in the area. They consist of coarse granular material with many subangular boulders, and their axes tend to lie in parallel and in an east–west direction. The ridges vary in length from 100 m to 800 m and in breadth from 75 m to 150 m. Their height above the level of the adjacent small lakes which often occur in the intervening hollows is 20–40 m. The south-facing slopes of the ridges are generally the steeper. The term *minor moraine* has been used by Lee, Craig, and Fyles (5) to describe this feature in adjacent Keewatin, and the "ripple till" of Ives (3) and others probably refers to this feature.

Elsewhere there are extensive areas of boulders, some large and angular and others smaller and rounded. The former are usually associated with bedrock, and Russell (9) interprets them as the result of frost or glacier action. He describes the deposits of rounded boulders as "the portion of the boulder till remaining after the finer constituents have been washed out" (ref. 9, p. 8).

The area of study lies to the immediate west of the transition from the Pre-Cambrian shield to the Hudson Bay Lowlands, and areas 20 km east of Caribou Lake show very immature drainage patterns with extensive deposits of deep peats overlying clays. This peat mantle is discontinuous and local in the Caribou Lake area, where the drainage is improved and where the mineral substratum is more granular, and is similar to the areas described (7) from an adjoining region to the south, at the confluence of Big Spruce River and Seal River.

Caribou Lake lies near the western limit of the marine inundation which followed the diminution of the last ice sheet (cf. Wright (13)) for it is here that beaches and spits are most common and they disappear further west. Russell (9) records marine fossils in a raised beach at Long Lake, 20 km ENE. from Caribou Lake. While no marine fossils were found during the present investigation, it would be difficult to explain the evidence of submergence in terms of a glacial lake rather than the sea, and it seems likely that the marine overlap extended as far as Caribou Lake.

At the junction of Caribou River with Caribou Lake there is a large deposit of sandy alluvium with a typical pattern of cut-off and slip-off slopes, oxbows, and meanders. There are a few large areas of outcropping bedrock in the vicinity of Caribou Lake, mainly Pre-Cambrian granites (Russell (9)).

## Vegetation

The physiographic types outlined above are of use as a framework for a description of the vegetation of the region. The main categories are: till and moraine, esker, beach and other sand deposits, alluvium, and peat. For each category three sorts of information will be provided: the vegetation will be described, the degree of association of vegetation with physiography will be indicated, and the photographic characteristics of the vegetation type will be enumerated.

*Till and Minor Moraine*

The greatest area of upland sites is occupied by a sparse, treeless vegetation which might be referred to the category tundra. However, in the immediate precincts of Caribou Lake abundant evidence was found that this tundra is of secondary status, covering areas which once bore forest. Many stumps and wind-felled trunks of spruce are characteristic of the area, indicating the presence of forest in the recent past. At present, a depauperate heath community grows on the thin, coarse mineral substratum of upland sites, made up of the following species, occurring together with monotonous regularity: *Loiseleuria procumbens*, *Empetrum hermaphroditum*, *Ledum decumbens*, *Vaccinium vitis-idaea* ssp. *minus*, *V. uliginosum*, *Cetraria nivalis*, *Cladonia rangiferina*, *C. mitis*, and *Alectoria altaica*; *Carex glacialis*, *C. bigelowii*, and *Luzula confusa* were noted on a few sites. The substratum is well drained, coarse in texture, and poorly developed. The upper 3 cm of dark-brown raw humus yielded an average (of six samples) pH reading[2] of 4.15. Under this layer is the coarse mineral material, with many boulders and stones (average pH value = 4.50).

The area of minor morainic ridges to the south of Caribou Lake illustrates the varied pattern of tundra and forest on upland sites. Certain ridges near the lake are definitely potential bearers of fair black spruce forest. For example, on the south slope of one ridge was recorded a regeneration forest community dominated by trees of *Picea mariana* with an average height of 7 m, diameter at breast height of 6.5 cm, and with 50 rings in the wood at breast height. The chief associated species recorded here were: *Betula papyrifera* var. *neoalaskana*, *B. glandulosa*, *Ledum groenlandicum*, *Alnus crispa*, *Larix laricina*, *Vaccinium vitis-idaea* ssp. *minus*, *V. uliginosum*, and *Empetrum hermaphroditum*. Elsewhere, ridges occur with smaller proportions of tree cover, with extensive areas occupied by a treeless heath community of prostrate shrubs. Between the ridges, and associated with small lakes and streams, are fragments of black spruce muskeg with occasional trees of larch and locally dense stands of tall willows, chiefly *Salix planifolia*. On a few south slopes, old gnarled trees, about 2 meters tall, of *Betula papyrifera* var. *neoalaskana* were observed. On the lower south-facing slopes in this area of minor moraine, a local community of shrubs was recorded with remarkable consistency. It consists of clumps of *Phyllodoce caerulea*, associated with *Kalmia polifolia*, *Vaccinium uliginosum*, *V. vitis-idaea* ssp. *minus*, *Empetrum hermaphroditum*, with occasional individuals of *Betula glandulosa*.

To the north of Caribou Lake a minor variant of this heathy tundra type was recorded, distinguished by the presence of cushions of the arctic species *Diapensia lapponica*, but showing no apparent habitat peculiarities. The substratum consists of coarse, gravelly material, with an upper layer of organic material. The average pH of six samples taken at 5 cm from the surface was

[2]All pH values cited in this report were determined in the field, or within about 4 hours of sampling, using a portable combination glass electrode meter.

5.15. The following species were recorded, the values shown indicating the average cover value[3] of 20-meter quadrats.

| | | | |
|---|---|---|---|
| *Betula glandulosa* | – 4 | *Carex bigelowii* | – + |
| *Empetrum hermaphroditum* | – 2 | *Luzula confusa* | – 1 |
| *Loiseleuria procumbens* | – 2 | *Rhacomitrium lanugiosum* | – 1 |
| *Vaccinium uliginosum* | – 3 | *Polytrichum piliferum* | – + |
| *Ledum decumbens* | – 3 | *Alectoria altaica* | – + |
| *Arctous alpina* | – 1 | *Cladonia mitis* | – 1 |
| *Diapensia lapponica* | – 1 | *Cetraria nivalis* | – 1 |

*Diapensia* grows in open situations where instability of the soil surface is apparent.

The heathy tundra of upland sites is not confined to drift and morainic deposits, but occurs commonly on esker and beach surfaces. On the aerial photographs its extent cannot be determined without making the assumption, probably valid, that it is a community exclusively of upland sites. In tone, it is the lightest of the closed vegetation types, being a light gray (N6–N7).[4] Local stands of dwarf birch and willow, for example on the lower slopes of certain morainic ridges where their presence is possibly controlled by protection of snow in winter, are gray (N5–N6) only slightly lighter than muskeg and scrub spruce forest. Areas of tundra lack any photographic texture. The exact boundary between this upland heathy tundra and low sedge tundra on peat is sometimes difficult to determine and almost impossible without stereoscopic viewing.

In the immediate area of Caribou Lake a large number of frost phenomena in coarse mineral soil (till) were noted, all of one main type. They consist of a roughly circular area of unstable, mineral soil, 3–8 m in width, with a level surface. The center of the "boil" consists of very fine, light-gray clay. These phenomena occur in till ridges and slopes, often at the margin of peat deposits.

The open, wet mineral soil of the center is being colonized by the following plants, which occur as scattered individuals: *Sagina caespitosa*, *Carex williamsii*, *Luzula confusa*, *Stellaria ciliatosepala*, and *Vaccinium uliginosum*. The average pH value for this soil is 7.3. Immediately surrounding this area is a shallow mantle of relatively undisturbed peat over mineral soil, bearing a vegetation dominated by *Ledum decumbens*, *Rubus chamaemorus*, *Vaccinium vitis-idaea* ssp. *minus*, *Cladonia rangiferina*, *Cetraria nivalis*, *Empetrum hermaphroditum*, and *Vaccinium uliginosum* (listed in order of relative abundance). The average pH value of this peat is 3.8. Other examples of this feature show more advanced colonization of the central zone, with a thin discontinuous layer of accumulating organic material. The plants recorded here were: *Loiseleuria procumbens*, *Carex bigelowii*, *Polytrichum piliferum*, *Empetrum hermaphroditum*, *Cetraria nivalis*, *Vaccinium uliginosum*, *Ledum decumbens*, *Cladonia rangiferina*, *Vaccinium vitis-idaea* ssp. *minus*, *Betula glandulosa*, and a small, squamule-bearing *Cladonia*.

[3]5 = covering 75–100%, 4 = covering 50–75%, 3 = covering 25–50%, 2 = covering 10–25%, 1 = covering less than 10%, + = single plants or shoots.
[4]The Munsell color chart notations are used here.

*Eskers and Beaches*

Detailed descriptions of the vegetation of esker surfaces at a locality about 50 km south of the present area have been provided elsewhere (7); the investigations in this area have yielded only a few additional or divergent points. In general, all eskers examined in the Caribou Lake area have been disturbed by human activity to a considerable extent. Evidence of old Indian camps and burial places was found on the several esker ridges examined, and it is likely that the original, natural vegetation of these sites has been drastically altered. In the Spruce Lake area, the most advanced vegetation type is a white spruce lichen forest. Here, no trees of *Picea glauca* were recorded on eskers, and only sporadic stands of black spruce, with a few small trees of white birch and certain very local groups of aspens represent the macrophanerophytic cover. A small esker at the east end of Caribou Lake consists of coarse mineral material, with many large rounded to subangular boulders in the surface. It is largely treeless with a poor lichen heath vegetation, similar to that described above for morainic ridges. The esker at the west end of Caribou Lake is made up of coarse sand, and much of the surface is in an unstable condition. Patterns of erosion and colonization similar to those described at Spruce Lake (7) are found here, and only a few minor differences are worthy of mention. In the southern part of the present area a high esker ridge was examined which exhibits remarkable relief, being approximately 90 m above the level of the surrounding terrain. There is a striking difference in the vegetation cover of the two slopes of this ridge; the south- and east-facing slope is covered by a continuous vegetation of *Betula glandulosa*, *Alnus crispa*, *Empetrum hermaphroditum*, *Arctous alpina*, *Vaccinium vitis-idaea* ssp. *minus* with various mosses and lichens and a few scrubby individuals of *Picea mariana* and *Betula papyrifera* var. *neoalaskana*. On the northwest-facing slope, which faces the prevailing wind and probably has little snow cover in winter, there is an open sandy gravel surface with scattered individuals of *Arabis arenicola* var. *pubescens*, *Artemisia canadensis*, *Potentilla nivea*, *Saxifraga tricuspidata*, *Festuca brachyphylla*, *Carex glacialis*, *Astragalus alpinus*, and *Vaccinium uliginosum*.

Rarely, on lower slopes of esker margins, segments of mature black spruce lichen forest were noted, showing close similarities to those described for Spruce Lake.

In this area, the vegetation types on esker surfaces are too fragmentary and small to be shown on any but a very large scale map, although they can be identified with ease on the photographs. The heathy tundra of stable esker surfaces is identical in appearance with that of till and moraine surfaces. Patches of lichen forest can be recognized on the esker flanks by the spaced, dark dots (trees) on a light-gray background, forming a coarse, open texture. Esker surfaces with continuous heath or shrub vegetation can be distinguished from open sand surfaces by their darker tone (N6–N7); open sand, on eskers and beaches, provides the lightest tone on the photographs (N8).

*Alluvial Deposits*

Where Caribou River enters Caribou Lake, there are extensive deposits of sand. This substratum bears a consistent zonation of vegetation, the zones showing some relation to the relative age of the deposits. The zones (Fig. 2A) present a change from an open community of submerged and emergent aquatics, through zones of sedge (zone II), pure willow scrub (zone III), mixed shrubs (IV), young conifers invading the shrub zones (zone V), an open forest of white spruce (zone VI), to a black spruce community on thick peat (zone VII).

The chief species of zone I are *Isoëtes muricata*, *Eleocharis acicularis* var. *submersa*, *Hippuris vulgaris*, *Colpodium fulvum*, and *Caltha natans*, all growing in 20–50 cm of water over siliceous sand. Zone II is a narrow (4–10 m) fringe of sedge, chiefly *Carex aquatilis*, abutting directly on the first terrestrial zone, zone III, which is dominated by tall individuals of *Salix planifolia* with a sparse ground vegetation (Table I) of *Calamagrostis canadensis* with the mosses *Polytrichum commune* and *Calliergon cordifolium*. The substratum consists of 3 cm of dark-brown fibrous humus (pH 4.80) over 6 cm of fine, light-brown silty sand with incorporated organic materials (pH 5.05); the underlying sand is coarse and red (pH 5.90). The mixed shrub zone (IV) is dominated by *Betula glandulosa*, *Alnus crispa*, and *Salix planifolia*, the main ground vegetation species being *Chamaedaphne calyculata* var. *angustifolia* and *Calamagrostis canadensis*. The substratum consists of 12 cm of light-brown organic material of fibrous texture, with fine silty sand throughout (pH 5.00), and 10 cm of silty sand, light brown except for humus staining (pH 5.50) over the underlying mineral material which is a sandy clay (pH 5.80). Zone V consists of a mixed shrub community of dwarf birch, alder, and willow, in which young trees of *Larix laricina* and *Picea glauca* are found. The larches

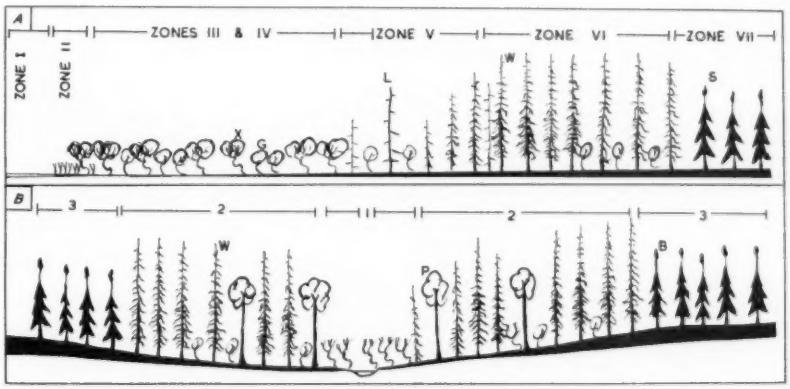


FIG. 2. Highly diagrammatic summaries of the vegetation zonation on alluvium at Caribou Lake (A), and along a small stream in till (B). The letters refer to dominant species, as follows: W, *Picea glauca*; S (B in B), *P. mariana*; P, *Populus balsamifera*; L, *Larix laricina*; X, *Salix planifolia*; and G, *Betula glandulosa*. In B, the zones 1, 2, and 3 are of willow, mixed spruce and poplar, and moss muskeg respectively (see text).

TABLE I

A cover analysis of zones III–VI of the community zonation on alluvium at Caribou Lake

| Species | Zone III | | | | | Zone IV | | | | | Zone V | | | | | Zone VI | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| *Polytrichum commune* | 5 | 4 | 1 | 2 | 1 | 1 | – | – | – | – | + | 3 | 2 | 3 | 1 | | | | | |
| *Myrica gale* | + | – | – | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Calamagrostis canadensis* | 2 | 2 | 2 | 1 | 2 | 3 | 3 | 3 | 1 | 2 | + | 1 | 1 | + | + | 1 | + | + | 1 | + |
| *Galium trifidum* | + | + | – | + | + | – | 1 | + | – | – | – | – | – | – | – | – | – | – | – | – |
| *Epilobium palustris* | + | + | – | – | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Calliergon cordifolium* | 1 | 2 | 1 | + | 1 | 1 | – | – | + | – | – | – | – | – | – | – | – | – | – | – |
| *Mnium punctatum* | + | + | 1 | + | 1 | + | + | – | + | + | – | – | – | – | – | – | – | – | – | – |
| *Vaccinium uliginosum* | + | – | – | + | + | + | + | 1 | 1 | + | 1 | 1 | – | + | 1 | + | 1 | 2 | 1 | 1 |
| *Potentilla palustris* | + | – | 1 | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Bare ground | – | – | 2 | 2 | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Chamaedaphne calyculata* var. *angustifolia* | – | – | – | – | – | 1 | 2 | 2 | 1 | + | 1 | 1 | 2 | 1 | + | – | + | – | + | – |
| *Ledum groenlandicum* | – | – | – | – | – | 1 | – | 1 | – | + | + | – | – | – | + | + | + | + | – | + |
| *Rubus chamaemorus* | – | – | – | – | – | + | 1 | 2 | 2 | + | 1 | 1 | 1 | – | 1 | 1 | 2 | 1 | + | + |
| *Sphagnum capillaceum* | – | – | – | – | – | 1 | 1 | 1 | + | 1 | 1 | + | 1 | 1 | 1 | – | – | – | – | – |
| *Mnium cinclidioides* | – | – | – | – | – | + | + | – | + | + | + | – | – | – | + | – | – | – | – | – |
| *Lycopodium annotinum* | – | – | – | – | – | – | + | – | + | – | – | – | – | – | – | – | – | – | – | – |
| *Peltigera aphthosa* | – | – | – | – | – | – | + | + | – | – | + | – | – | – | + | 1 | + | + | – | 1 |
| *Hypnum lindbergii* | – | – | – | – | – | – | – | – | + | + | – | + | 1 | 1 | + | – | 1 | + | – | – |
| *Vaccinium vitis-idaea* ssp. *minor* | – | – | – | – | – | – | – | – | – | – | 1 | + | + | 1 | + | 2 | 1 | 1 | + | 1 |
| *Sphagnum fuscum* | – | – | – | – | – | – | – | – | – | – | + | – | 1 | 2 | 1 | + | 1 | 1 | 2 | 1 |
| *Cornus canadensis* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 2 | + | + | 1 | + |
| *Empetrum hermaphroditum* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | + | + | 1 | + | + |
| *Aulacomnium palustre* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | + | + | + | + | + |
| *Ledum decumbens* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | + | + | 1 | + |
| *Sphagnum warnstorfianum* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | + | 1 | + | 1 | 1 |
| *Dicranum bergeri* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 1 | – | + | – |
| *Drepanocladus fluitans* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 1 | – | + | – |
| *Tetraplodon angustatus* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | + | – | – |

vary from 9 m height, 8 cm diameter at breast height (d.b.h. hereafter), and 57 years age, to 2.5 m, 2 cm at 13 years. White spruce were recorded from 12 m, 18 cm d.b.h. at 103 years to 6 m, 7 cm d.b.h. at 57 years. Associated with the conifers is an open shrub layer, 1–2 m in height, composed of dwarf birch, alder, and willow, and a low shrub layer of *Ledum groenlandicum*, *Vaccinium uliginosum*, and *Chamaedaphne calyculata* var. *angustifolia*. The substratum consists of 2 cm of mohr (pH 4.0), 7 cm of wet moss peat with some fine mineral material (pH 4.85), overlying a clay loam substratum of light-brown color with local humus staining dropping out below (pH 5.0). There is no apparent profile development. A white spruce forest forms zone VI, made up of an uneven aged, open stand of white spruce, associated with occasional trees of larch. The density of the forest here has been influenced by sporadic logging activities during the period when there was a Hudson's Bay Post at Caribou Lake. The spruces varied from 16 m, 28 cm d.b.h. at 265 years to 7 m, 15 cm d.b.h. at 58 years. The ground vegetation is summarized in Table I. The substratum consists of 12 cm of black, fibrous humus (pH 4.60) over a gleyed clay loam of mottled gray and red color (pH 5.10). Permafrost at the end of July was found at 45 cm.

The last zone (VII) consists of an open cover of *Picea mariana*, the trees being 7 m tall, 15 cm d.b.h. at 55 years. The substratum consists of 24 cm of dark-brown fibrous peat (pH 4.00) over a mineral soil of light-brown clay with sand (pH 4.35). Permafrost (late July) was found at 24 cm.

Two small, fragmentary stands of forest on alluvium were noted on the north and south shores respectively of Caribou Lake. These stands of white spruce (*Picea glauca*) show interesting floristic resemblances to forests characteristic of the boreal regions rather than the subarctic. The first is on the south shore of the eastern arm of Caribou Lake and consists of a closed stand of white spruce with a few old, decadent trees of *Populus balsamifera*. The tallest spruces reach 20 m with d.b.h. of 65 cm and an age between 250 and 300 years. (The age of the oldest trees could not be ascertained precisely because of rot in the centers of the trunks.) The stand is of uneven age, but with very few sapling trees. A discontinuous tall medium shrub stratum was recorded throughout the stand, consisting of *Salix planifolia*, *Viburnum edule*, *Ribes triste*, *Rubus idaeus* var. *strigosus*, and *Betula glandulosa*. The minor vegetation was noted as follows:

| | | | |
|---|---|---|---|
| *Equisetum sylvaticum* | – 3 | *Viola palustris* | – 2 |
| *Rubus acaulis* | – 3 | *Calamagrostis canadensis* | – 2 |
| *Lycopus uniflorus* | – 3 | *Mnium cuspidatum* | – 2 |
| *Dryopteris disjuncta* | – 3 | *Hypnum lindbergii* | – 2 |
| *Pyrola asarifolia* | – 2 | *Calliergon cordifolium* | – 2 |
| | | *Carex vaginata* | – 1 |

The substratum consisted of a moderately well drained light-textured soil with slight profile development, as follows:

A, 2–3 cm loose, fibrous humus (pH 5.5);

A, 4–5 cm compacted black fibrous humus with mineral particles;

BC, 18 cm of light-brown silt–loam with coarser material below pH 5.90.

Through the middle of this small stand a stream flows, and there is a broad zonation of vegetation as one passes away from the stream. Immediately abutting the stream is a willow fringe, followed by the mixed spruce–poplar stand described above, followed by a fragmentary stand of pure white spruce with the ground vegetation dominated by "feather mosses", mainly *Pleurozium schreberi* and *Hylocomium splendens*, and this abuts on to moss muskeg on shallow wet peat. These zones have been summarized diagrammatically in Fig. 2B.

The second stand of white spruce occurs along a small stream flowing into the north side of Caribou Lake. Here there is a narrow strip of tall white spruce along the stream channel, surrounded on both sides by moss muskeg on peat. The spruces are relatively tall, and symmetrical, the average values of mature trees being 20 m, 38 cm d.b.h. at 110 years. A tall willow stratum of *Salix planifolia* occurs at 2 m. The following list was compiled from the ground vegetation:

| | | | |
|---|---|---|---|
| *Equisetum sylvaticum* | – 3 | *Ribes triste* | – 2 |
| *Rubus acaulis* | – 3 | *Galium trifidum* | – 2 |
| *Carex brunnescens* | – 3 | *Hylocomium splendens* | – 1 |
| *Calamagrostis canadensis* | – 2 | *Lycopodium annotinum* | – 1 |
| *Calliergon cordifolium* | – 2 | *Moneses uniflora* | – 1 |
| *Betula glandulosa* | – 2 | *Viola palustris* | – 1 |
| *Hypnum lindbergii* | – 2 | *Linnaea borealis* var. *americana* | – + |
| *Peltigera aphthosa* | – 2 | *Mnium cuspidatum* | – + |
| *Vaccinium vitis-idaea* ssp. *minus* | – + | *Ribes glandulosum* | – + |

The substratum consists of 40 cm of wet muck over a fine silty sand (pH of the upper layer, 5.80).

*Peat Deposits*

Three main vegetation types occur on peat—moss muskeg,[5] heath bog, and sedge–cottongrass bog. Fens are absent from the region.

*Moss muskeg* is confined to shallow peat (30–80 cm) in basins. The vegetation consists of an open stand of poorly grown *Picea mariana*, associated with small ericoid shrubs (*Ledum groenlandicum*, *Vaccinium vitis-idaea* ssp. *minor*, *V. uliginosum*, *Andromeda*) and a ground cover of mosses (chiefly *Sphagnum fuscum*, *S. warnstorfianum*, *S. capillaceum*, and *Aulacomnium palustre*). In subarctic Manitoba as a whole the floristic and physiognomic features of moss muskeg vegetation remain remarkably constant.

*Heath bog* and *sedge–cottongrass bog* are found on certain conspicuous peat deposits, described elsewhere (7). They consist of thick, sometimes stratified mantles of peat, occurring here as residual pockets along lake shores. Field work, and the scrutiny of aerial photographs of all of northern Manitoba, reveal that these phenomena are of general occurrence in the subarctic zone. Most of them are slowly eroding into the abutting lakes, exposing vertical faces of peat which calve into the water in large slabs. The surfaces of the deposits show extensive polygonal fissures. Several peats were excavated at

[5]The terms used here to describe peat vegetation are defined in ref. 8.

Caribou Lake and sampled for subsequent stratigraphical and pollen analytical work. The vertical faces revealed a fairly consistent stratification of five thin (1–2 cm) fibrous layers alternating with five broader (15–25 cm) layers of light-brown *Sphagnum* peat. The lowest peat stratum was fibrous and contained numerous large fragments of spruce; the upper 10–15 cm was of black fibrous peat. The chief macrofossils in the fibrous peat were of ericaceous shrubs and Glumiflorae. Pollen analysis is being conducted currently in the hope that some light will be shed on the nature and origin of the deposits, and possibly on the postglacial changes in vegetation of the area. Under the peat is a fine, light-gray, slightly calcareous clay.

The vegetation of these peat surfaces is closely similar to that described (7) for the neighboring Seal River area. The drier peats bear a heath vegetation, dominated by *Ledum decumbens*, *Vaccinium vitis-idaea* ssp. *minor*, and *Empetrum hermaphroditum*, while the wetter are covered by a meadow type made up mainly of *Carex paupercula*, *C. capitata*, *Eriophorum spissum*, and *Scirpus caespitosus* ssp. *austriacus*.

On the aerial photographs, moss muskeg is dark gray (N4) in tone, and shows fine stippling; individual trees are not always visible. Heath bog is gray in tone (N5) and sedge–cottongrass bog is light gray to white (N7–N8). Neither shows photographic textures, but two features of value in recognizing these types on deep peats are the network of fissures and the presence of small round, shallow ponds. Also, these deep peats are invariably close to lakes or on islands.

## Floristic Notes

The flora of the area is impoverished and monotonous. The list of vascular plants is meager; in the precincts of Caribou Lake only 115 species were recorded. Likewise, Scoggan (11) could find only 85 species in the forest–tundra regions round Baralzon and Nejanilini Lakes, approximately 100 km northwest of Caribou Lake. Of these, only the following 20 are exclusively arctic (often low-arctic) species (those marked with an asterisk were not recorded at Spruce Lake (7), 50 km south of Caribou Lake): *Colpodium fulva*, *Hierochloe alpina**, *Carex stans*, *C. bigelowii*, *C. glacialis*, *C. capillaris* ssp. *arctogena*, *Juncus castaneus**, *J. albescens*, *Luzula confusa*, *Stellaria ciliatosepala*, *Sagina caespitosa**, *Arabis arenicola*, *Potentilla nivea**, *Ledum decumbens*, *Arctous alpina*, *Phyllodoce caerulea*, and *Diapensia lapponica**. The other vascular plant species, making up about 85% of the flora, are wide-ranging boreal or boreal–arctic species. The flora is very similar to that described in greater detail for Spruce Lake (7) and it corroborates the view (7, p. 4) that there is a striking floristic difference between adjacent climatically equivalent areas in Manitoba on the Pre-Cambrian shield and on the Hudson Bay Lowlands. These findings are similar to those of Hustich (1, 2) for areas in northern Ontario.

Of some interest is the fact that *Phyllodoce caerulea*, often described as a rare species, was recorded in local abundance in several localities near Caribou

TABLE II

A guide to the identification of forest–tundra vegetation types on vertical aerial photographs

| Vegetation type | Tone | Texture | Appearance | Associated physiography |
|---|---|---|---|---|
| Upland tundra (heath) | Gray – light gray (N6–N7) | Smooth | Featureless; esker surfaces show local crenate patches (of heath vegetation) | Till, washboard moraine, esker, and beaches |
| Heath bog | Gray – light gray (N6–N7) | Smooth, broken only by lakes and patterned ground | Flat surface, often with fine reticulate patterns; eroding faces abutting on lakes often visible; often small round ponds and lakes in the peat | Low areas of till, usually associated with lakes |
| Moss muskeg | Dark gray (N4) | Fine stippling | Individual trees not always visible; often diffuse | Depressions in till and moraine, on abandoned channels and older alluvium |
| Closed conifer forest on alluvium | Dark gray (N4) | Fine to coarse stippling | Tallest, spire-shaped spruces forming a palisade along banks of river; smaller squat trees away from banks | Alluvium; recognized by the absence of relief, presence of meanders, oxbows, swales, and flats |
| Open conifer forest | Dark-gray spots on light-gray background (N4 on N6–N7) | Smooth, broken only by trees | Individual trees visible, well spaced; spire-shaped. | Eskers, washboard moraine, and beaches |
| Shrub | Gray (N5–N6) | Smooth | Featureless; rarely individual clumps are visible | Lower slopes of moraine ridges, edges of watercourses; young alluvium |
| Sedge–cottongrass bog | Light gray to white (N7 to N8) | Smooth | Flat surface, rarely patterned by parallel ridges (Strängmoor) with intervening open water | Depressions |

Lake. It occurs most abundantly, and almost exclusively, on the steeper, south-facing slopes of minor moraine ridges, where it is likely that greater snow accumulation provides some protection (cf. ref. 6, p. 132).

The prevalent peat and coarse, acid mineral substrata yield very few species, and only in rare, local sites is it possible to discover any diversity of flora. For example, in frost "boils", the unstable, fine clay substratum with neutral reaction bears a few of the more interesting floristic records, such as *Carex williamsii*, *Stellaria ciliatosepala*, *Juncus albescens*, and *Sagina caespitosa*.

## Concluding Comments

The loss of data and materials by accident has reduced the value of this particular investigation, and the main reason for submitting this brief report for publication is to make available a preliminary conspectus on the vegetation of a region remarkable for the paucity of botanical information.

The configuration of vegetation types in the appended map (Fig. 3) is reasonably typical of the forest–tundra zone as a whole, as more extensive studies (8) have shown. Within the zone, changes in the relative amounts of major community types appear to be controlled by changes in physiography and by historical factors. In Table II, a summary is provided of the main vegetation types of the area, with some notes on the associated physiography and the usual appearance of the vegetation on high-altitude, vertical aerial photographs.

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NOTE: Fig. 3 follows.

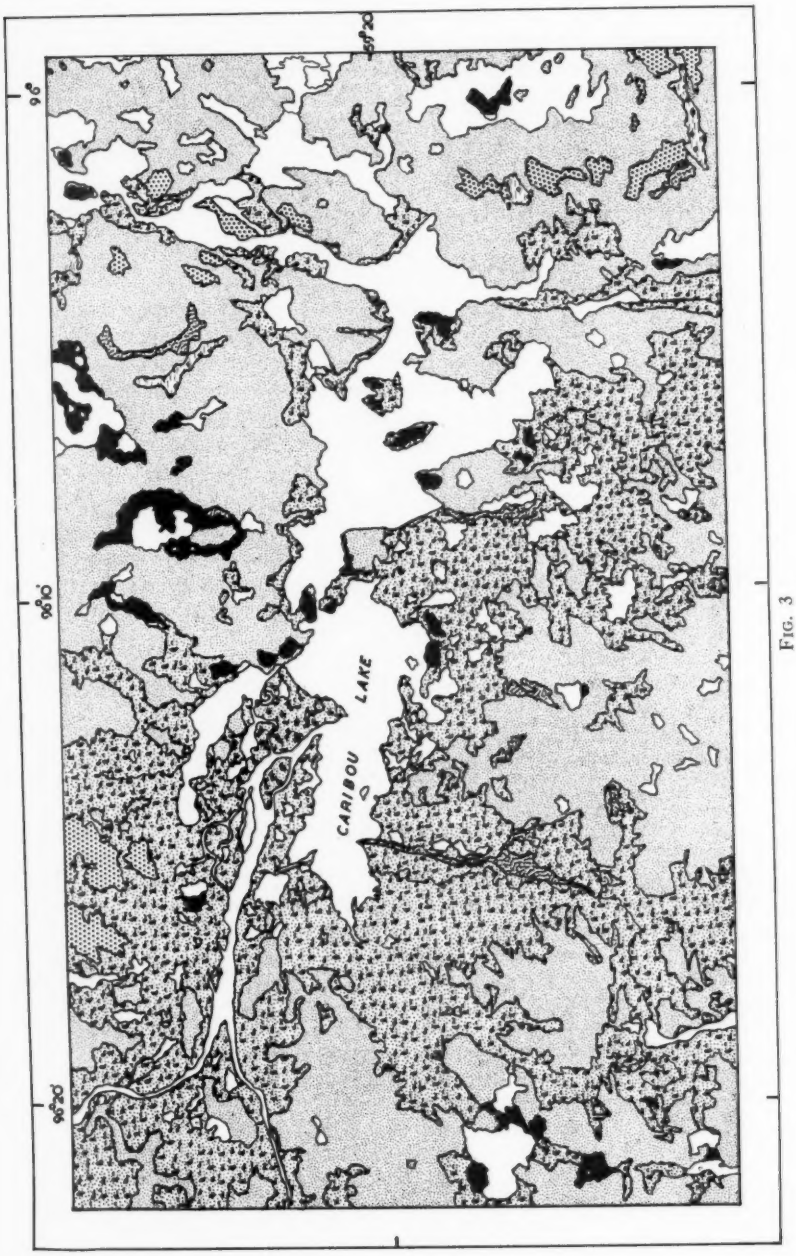


FIG. 3

FIG. 3. A vegetation map of the Caribou Lake area (scale 1:120,000). Unshaded areas of the map depict open water.

Areas of black spruce forest, predominantly moss muskeg on peat, but locally of scrub forest on moraine.

A complex of moss muskeg and white spruce and shrub communities on alluvial deposits.

Heath tundra and areas of dwarf birch and willow scrub on upland sites of moraine.

Heath or sedge–cottongrass tundra on deep peat deposits.


Wet sedge–cottongrass bog on shallow, poorly drained peats.

Areas of more or less bare sand or gravel.


Areas of outcropping bedrock.

In the absence of base maps of this region, the vegetation map was compiled by mapping directly on enlargements (twice) of the vertical aerial photographs and transferring to the present map with a reducing pantograph.

# EFFECT OF 3-(4-CHLOROPHENYL)-1,1-DIMETHYLUREA ON DRY MATTER PRODUCTION, TRANSPIRATION, AND ROOT EXTENSION[1]

WM. HAROLD MINSHALL

## Abstract

Extension growth of the chlorophyll-containing roots of *Hydrocharis morsus-ranae* was inhibited by 0.5 p.p.m. of 3-(4-chlorophenyl)-1,1-dimethylurea (monuron) whereas concentrations close to the water saturation point of 230 p.p.m. were required to inhibit extension growth of the non-chlorophyll-containing attached roots of *Zea mays* and *Phleum pratense* and the detached roots of *Pisum sativum*.

A total of 15–20 μg of monuron per gram fresh leaf applied through the cut petiole of detached primary leaves of *Phaseolus vulgaris* inhibited the increase of dry matter by 90% and suppressed transpiration 40–50%. Internal concentrations of 1–2 μg/g of monuron produced simultaneous enhancement of dry matter increase and of transpiration but concentrations of 5–10 μg/g produced a suppression of dry matter increase concurrently with an enhancement of transpiration. Age of leaf and the time of year in which the plants were grown altered the critical internal concentration levels required to affect dry matter increase and transpiration.

Analysis of detached leaves treated with 15–20 μg/g monuron indicated a marked suppression of the formation of non-water-soluble carbohydrate, a slight suppression of the formation of water-soluble nitrogen, but little or no effect on water-soluble carbohydrate or on non-water-soluble nitrogen.

In detached leaves *o*-phenanthroline, 3-phenyl-1,1-dimethylurea, and 3-(3,4-dichlorophenyl)-1,1-dimethylurea resembled monuron closely in symptom development and in their effect on dry matter production and transpiration. Iodoacetamide, 2,4-dinitrophenol, and 8-hydroxyquinoline each produced some effects similar to monuron but differed from it in certain respects. Thiourea, sodium diethyldithiocarbamate, sodium fluoracetate, ethyl-NN-diphenylcarbamate, and hydroxylamine hydrochloride were without noticeable effect.

## Introduction

Light has been found to affect the degree and type of symptoms produced in plants by the herbicidal chemical 3-(4-chlorophenyl)-1,1-dimethylurea (monuron) (14, 16). The effects of light suggested that the site of the toxic action of monuron might lie in the light-dependent process of photosynthesis. From investigations of the effect of monuron on dry matter increase in detached bean leaves and on the extension growth of frogbit roots it was evident that this chemical inhibited dry matter production with an accompanying decrease in the rate of transpiration. A preliminary summary has been published elsewhere (15) and some of the material was presented before the American Society of Plant Physiology at Stanford, California, in August, 1957, and to the Weed Society of America at Memphis, Tennessee, in January, 1958.

## Materials and Methods

### *Detached Leaves*

Detached primary leaves of the kidney bean (*Phaseolus vulgaris* L. var. Black Valentine) proved to be excellent material for these studies. Yarwood

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(19), who compiled a list of 322 references covering the use of detached leaves, has pointed out that this type of culture is an old and well-established method of experimentation and that most of our early knowledge of photosynthesis came from such studies. Dry matter increase in plants is the result of photosynthetic action and the rate of this increase per unit of leaf area is a measure of the excess of photosynthetic rate over the rate of dry matter loss by respiration. When detached from the plant the leaf becomes an isolated organ and transport away from the leaf is prevented. When held on water detached primary leaves of bean were found to retain a healthy green color for 12 to 14 days and their increase in dry weight was approximately linear for 5 to 7 days (Fig. 3). As bacterial contamination did not develop in the flasks holding the leaves it was not necessary to remove additional slices from the petiole at intervals during the experiment. The lack of microorganism development in the flasks was believed to indicate that leakage of water-soluble materials from the petioles was either non-existent or of a low order.

In the present as well as in previous studies (16) the type of symptoms that developed from monuron treatment in detached leaves was similar to those that developed in intact plants, indicating that the toxic action of monuron in detached leaves is similar to that in attached leaves.

Weekly plantings of beans in 4-in. clay pots of soil in the greenhouse provided a continual source of primary leaves for these investigations. In the summer months slatted shades on the exterior of the greenhouse reduced the maximum intensity of sunlight from 10,000 to 3,000 ft-c and moderated the temperature. Other than the shades, however, plants were subject to ordinary greenhouse conditions without supplementary light. In the greenhouse, mature Black Valentine beans consisted of one pair of primary leaves and either three or four trifoliate leaves. By observation it was noted that the lamina of the primary leaves continued to expand until the first trifoliate leaf was from one quarter to one half of its ultimate size. It was considered advisable to detach the primary leaf from the bean plant soon after it had reached full size, that is, at a time when the assimilation rate was largely dependent on external conditions but before the efficiency of photosynthesis was markedly influenced by "aging" factors within the leaf. For this reason the primary leaves were detached from the plants when the first trifoliate leaf was approximately half expanded, except, of course, in those experiments wherein the effect of age of leaf on monuron action was under investigation. The primary leaves were detached under water with the cut made across the petiole some 2–3 mm above the lower pulvinus which joins the leaf to the stem. As soon as the leaf was detached the cut end of the petiole was placed in a small flask of distilled water. All experiments with detached leaves were carried out in an air-conditioned laboratory under a panel of 14, 96-in., slimline, standard cool white fluorescent lamps at a light intensity of 1200 ft-c, a temperature of 80° F, and a relative humidity of 40%. To obviate the daily rhythm of movement at the upper pulvinus and to make certain that all leaves were subject to a uniform light intensity during the experiment

the blades of the detached leaves were held parallel to the fluorescent lamps with glass rod supports.

In the early stages of these investigations different internal dosages were obtained by exposing the petioles of the detached leaves to a single external concentration for different time intervals. Because of the variation in water uptake from leaf to leaf and from season to season, desired internal concentrations of chemical were difficult to achieve and for this reason the "time" method was abandoned in favor of a "weighing" method. The leaf was weighed when detached from the plant and from prepared tables a quick calculation was made of the amount of stock solution that must be taken up to give the desired internal concentration. Frequent weighings were then made of the solution and when uptake had reached the calculated level the treated leaf was transferred from the test tube of stock solution to a flask of water. When the leaf was detached from the plant the lumen of the petiole was filled with air but by the day following the excision the solution in which the petiole was standing had usually entered the lumen. Once the solution had entered the lumen of the petiole, selective absorption through petiole cells would be possible. By sealing off the cut ends of the petioles with a low-melting-point wax it was found that the lumen of the petiole remained filled with air for several hours after detachment and the solution in which the leaf was standing entered the open ends of the water-conducting vessels. It was possible, therefore, to calculate the internal concentration of the chemical from the amount of stock solution taken up. In most of the experiments the uptake period was less than three hours and the stock solutions of monuron were between 25 and 128 p.p.m.

Frequently a few leaves wilted following detachment from the plant and in the 50 experiments carried out involving more than 2000 detached leaves extensive wilting occurred in nine experiments. Young leaves were more prone to wilt than were older leaves. As leaves detached at midday or in the afternoon were more prone to wilt than were those detached in the morning, the plants to be used in an experiment were carried to the laboratory either the night previous to or in the early morning of the day of the experiment and were held in 50 ft-c of light until the leaves were detached. Since wilting stops the photosynthetic process immediately, unless otherwise stated, only those experiments are included in which less than 20% of the leaves wilted and in which the degree of wilting was slight followed by rapid recovery. Monuron was without effect on the wilting of detached bean leaves.

*Roots*

Root extension growth was investigated in a series of monuron concentrations at room temperature with frogbit (*Hydrocharis morsus-ranae* L.), timothy (*Phleum pratense* L.), corn (*Zea mays* L., hybrid, Pfister 44, MF.), and detached roots of pea (*Pisum sativum* L. var. Perfection).

Young clonal frogbit plants consisting of two leaves, two roots, and a bud were grown for 7 days in $\frac{1}{4}$-gal glazed crocks under 600 ft-c continuous light

from fluorescent lamps. Monuron, 70% wettable powder, was added to the nutrient solution described elsewhere (17).

Five detached root tips of pea, 1 cm in length, were cultured in the dark for 7 days on 0.5% agar in 10-cm petri dishes. Monuron, 70% wettable powder, was added to the medium of Bonner and Devirian (3).

Tests with timothy and corn were made in water solutions of monuron, 99.9% pure. Twenty seeds of timothy were floated on 100 ml of solution in a 125-ml Erlenmeyer flask in the dark. The seeds germinated on the third day and root lengths were measured on the fifth day. Ten corn seedlings with radicles from 1 to 4 cm long were grown for 48 hours on cheesecloth platforms in squat preparation dishes containing approximately 250 ml of solution. The cheesecloth platforms were stretched over glass cylinders 1 in. in height and were held in place with elastic bands. The dishes were held in 150 ft-c of light from fluorescent lamps and the water lost by evaporation was replaced daily.

*Measurement and Analysis*

The amount of water lost by transpiration was determined daily by weighing. Dry weights of leaves were determined after drying at 70° C in a vacuum oven. Leaf areas were measured from contact photographs with a planimeter. Except where noted otherwise, the monuron used in these investigations was recrystallized from the commercial product to a purity of 99.9%. Stock solutions of monuron were prepared by first dissolving the pure chemical in acetone. Then, while rapidly stirring, the required amount of the acetone solution was dispensed with a pipette into a measured quantity of double

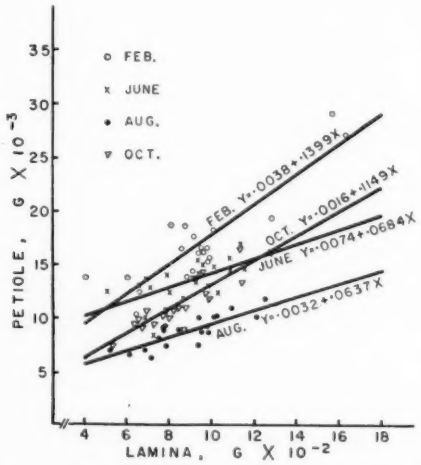


FIG. 1. The relationship between dry weight of lamina and dry weight of petiole of primary leaves of bean. Correlation coefficients for the 20 leaves detached from plants grown in different months were: Feb. 0.8967; June 0.6269; Aug. 0.8416; and Oct. 0.8845.

distilled water. Air was bubbled through these stock solutions for at least 48 hours to lower the concentration of acetone well below its toxic level.

Although dry weights of petioles and of blades were determined separately in the different experiments they are combined in the present paper. When the correlation coefficients from the scatter diagram (Fig. 1) of the dry weights of blades and petioles at the time of removal from the plant were compared with Table 6 of Fisher and Yates (7) it was found that leaves detached from plants grown in February, August, or October had correlation coefficients significant at the .001 level and leaves detached from plants grown in June at the .01 level. Furthermore, the source of the increase in the weight of the petiole of the detached leaf was found to arise from within the blade. There is, therefore, ample justification for treating the blade and petiole as a single system.

Changes in nitrogen and carbohydrate were followed in some of the detached leaf cultures. The dried leaves were ground in an intermediate Wiley mill to pass a 60-mesh sieve, extracted with water, and the determinations carried out on the extract and the residue. Nitrogen was determined by the Kjeldahl method and carbohydrate by the anthrone method (12).

## Results

### *Dry Matter*

Internal concentrations as low as 15–20 μg monuron per gram fresh leaf inhibited the dry matter increase by 90% in primary leaves of bean detached from plants grown in August (Fig. 3). Higher internal concentrations inhibited the dry matter increase to an even greater extent. The suppression of dry matter increase was not as great in older leaves in which the photosynthetic capacity was declining as it was in leaves that had just reached maturity (Table I); or was the suppression as great in leaves detached from plants grown in February or October as it was in leaves from plants grown in June or August (Table II). When the carbohydrate content of the leaves was

TABLE I

Effect of different concentrations of monuron per gram fresh leaf on the dry weight increase in 4 days of leaves detached from 3- and 4-week-old bean plants. Seeds were planted May 24

| Treatment | Number leaves | Mean dry weight with standard error, mg/cm² | Change in dry weight, mg/cm² | Increase in dry weight, % |
|---|---|---|---|---|
| | | 3-week-old leaves | | |
| Zero-time control | 10 | 1.52 ± .043 | — | — |
| 0 monuron (water control) | 10 | 3.16 ± .055 | +1.64 | 100 |
| 20 ± 0.5 μg/g monuron | 15 | 1.64 ± .055 | + .12 | 7 |
| 39 ± 0.6 μg/g monuron | 15 | 1.45 ± .037 | − .07 | 0 |
| | | 4-week-old leaves | | |
| Zero-time control | 10 | 1.68 ± .033 | — | — |
| 0 monuron (water control) | 10 | 2.92 ± .063 | +1.24 | 100 |
| 23 ± 0.6 μg/g monuron | 15 | 1.85 ± .052 | + .17 | 14 |
| 40 ± 0.4 μg/g monuron | 15 | 1.63 ± .037 | − .05 | 0 |

TABLE II

Effect of approximately 20 μg monuron/g fresh leaf on the increase in dry weight of primary leaves detached from bean plants grown at different times of the year. When the primary leaves were detached the first trifoliate leaf was approximately half size in all experiments

| Date leaves detached | Age from planting of seed, days | Concentration of munoron in leaf with standard error, μg/g | Number leaves | Duration of detached leaf test, days | Average dry weight with standard error, mg/cm² | Average dry weight for a 5-day period (lines 1, 2, 3, 4 estimated from previous column), mg/cm² | Increase in dry weight in 5 days, mg/cm² | Increase in dry weight, % |
|---|---|---|---|---|---|---|---|---|
| Feb. 17 | 45 | 0 | 10 | 7 | 4.94±.110 | 4.50 | 1.75 | 100 |
| | | 21±1.6 | 20 | 7 | 3.64±.130 | 3.60 | .85 | 49 |
| June 14 | 21 | 0 | 10 | 4 | 3.16±.055 | 3.40 | 1.88 | 100 |
| | | 20±0.5 | 15 | 4 | 1.64±.055 | 1.64 | .12 | 6 |
| Aug. 17 | 18 | 0 | 10 | 5 | 2.93±.046 | 2.93 | 1.65 | 100 |
| | | 18±0.8 | 10 | 5 | 1.39±.051 | 1.39 | .11 | 7 |
| Oct. 12 | 25 | 0 | 10 | 5 | 4.09±.101 | 4.09 | 2.24 | 100 |
| | | 24±0.8 | 10 | 5 | 2.15±.138 | 2.15 | .30 | 13 |

TABLE III

Effect of 0, 18, and 50 μg monuron/g fresh leaf on dry weight, nitrogen content, and carbohydrate content of detached bean leaves. The leaves were detached Aug. 17 from 2½-week-old plants. Standard errors are included for certain values

| Treatment | Number leaves | Duration of detached leaf test, days | Av. area, cm² | Av. dry weight, mg/cm² | Nitrogen | | Carbohydrate | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Water soluble, mg/cm² | Water insoluble, mg/cm² | Water soluble, mg/cm² | Water insoluble, mg/cm² |
| Zero-time control | 20 | 0 | 75±2.7 | 1.28±.074 | .019 | .085 | .027 | .113 |
| Water control | 10 | 5 | 74±5.0 | 2.93±.046 | .036 | .078 | .028 | 1.424 |
| 18±0.8 μg/g monuron | 10 | 5 | 72±3.9 | 1.39±.051 | .024 | .079 | .019 | .118 |
| 50±1.2 μg/g monuron | 10 | 5 | 70±3.1 | 1.18±.019 | .023 | .064 | .016 | .083 |

determined (Table III) it was evident that while monuron reduced slightly the water-soluble fraction of carbohydrate, it very markedly suppressed the increase in the water-insoluble fraction, presumably starch, which developed in the water control leaves. This drastic suppression of the production of photosynthate indicates that monuron was poisoning photosynthesis.

Although an amount equivalent to 15–20 μg/g was required to approach complete suppression of dry matter increase in detached leaves the critical internal concentration of monuron for poisoning photosynthesis may be less than this amount. In the experiment illustrated in Fig. 3 the leaves which absorbed 17 μg/g monuron developed acute water-soak blotch in 1% of their marginal areas. This blotch is a symptom shown in a previous paper (16) to require an internal concentration in excess of 100 μg/g. Evidently, the monuron entering the leaf by the petiole was not distributed evenly throughout the entire blade. In all probability, therefore, the critical internal concentration for inhibition of photosynthesis on a cellular level will be less than 15 μg/g.

*Nitrogen Metabolism*

From determinations of the nitrogen fraction of detached leaves (Table III) it was found that the leaves held on water exhibited the well-known changes

described by Chibnall (4) and Bonner (2, Chap. 20) of a decrease or breakdown of water-insoluble nitrogen (protein) and an increase in water-soluble nitrogen (amino acids). When detached leaves were treated with monuron the insoluble fraction decreased to approximately the same level as in water control leaves and the soluble fraction had increased slightly but not as much as had occurred in the water control leaves. These data suggest that while monuron had little if any effect on the breakdown of proteins within the detached leaves it may have suppressed somewhat the synthesis of the water-soluble constituents or amino acids.

In symptomatological studies (16) it was noted that occasionally mature leaves of bean treated with 25 $\mu$g/g or more of monuron rapidly turned yellow in color whereas on other, rarer occasions, leaves treated with monuron were protected from the customary yellowing that usually developed in 7–10 days after detachment. Michael (13) has shown that protein hydrolysis of the cytoplasmic and chloroplastic fractions takes place concurrently and the decomposition of the protein–chlorophyll complex leads to the loss of chlorophyll and to the yellowing of detached leaves. From the symptomatological studies it would appear that, depending on internal concentration, age of leaf, and other conditions not known, monuron treatment may either prevent or enhance the decomposition of the protein–chlorophyll complex. In the experiment where leaves were analyzed for nitrogen and described above neither enhancement nor protection from yellowing was noted in the monuron-treated leaves.

The possibility that the changes in nitrogen metabolism occurring in detached shoots might affect the action of monuron in leaves detached from these shoots was investigated in two separate experiments. When primary leaves, removed from detached shoots that had been held on water for 3 days and in which the insoluble nitrogen had decreased from 6.3% to 4.6%, were treated with monuron the development of symptoms, the inhibition of dry matter increase, and the partial suppression of transpiration were the same as in similarly treated leaves from intact plants. It is believed, therefore, that the effects on protein metabolism are secondary effects and do not form an essential part of the primary toxic action of monuron.

*Transpiration*

By the day after treatment internal concentrations of 15–20 $\mu$g monuron per gram fresh leaf detached from plants grown in June or August reduced the transpiration by 40 to 50% of that of the water controls (Fig. 5). The transpiration then remained close to this reduced level for 3–4 days when it usually increased slightly. As with the effect of monuron on dry matter increase, the extent of the suppression of transpiration varied somewhat depending on age of leaf, time of year in which plants were grown, etc. The suppression of transpiration was evident within one hour from the start of treatment with monuron.

*Root Extension*

Corroborative evidence for the photosynthetic poisoning action of monuron is available from its effect on the extension growth of the green roots of frogbit. To suppress severely the extension growth of roots of corn and timothy, water solutions close to the saturation point of 230 p.p.m. were required (Fig. 2). The dosage response of detached roots of pea was approximately the same as that of attached roots of corn and timothy, indicating that the toxic action was exerted directly on the attached roots. Extension growth of frogbit, however, is suppressed by a concentration 1/500 of that required to suppress corn, timothy, or pea (Fig. 2). Frogbit, a floating hydrophyte, differs from the other three mesophytes in that its roots contain chlorophyll. It has been shown elsewhere (17) that photosynthesis is necessary within each root for normal root extension growth to occur. Anything interfering, therefore, with the photosynthetic reaction in frogbit roots will suppress extension growth of the roots. Monuron apparently poisons the photosynthetic reaction in frogbit roots and as a result external concentrations of 0.5 p.p.m. markedly suppress root extension growth. Roots of corn, timothy, and pea, however, do not contain chlorophyll and, therefore, the photosynthetic poisoning action of monuron cannot exert a direct effect on the root extension growth of these species.

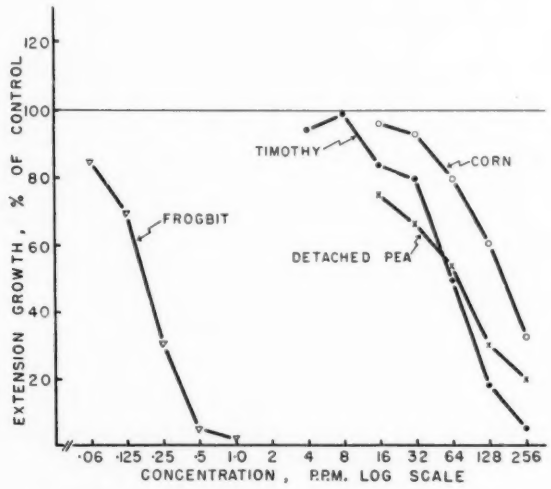


FIG. 2. The effect of a range of monuron concentrations on the dosage-response of root extension growth. All points are average values given as percentage of control cultures. The number of experiments and average extension growth of the control cultures were as follows: frogbit—six experiments with single clonal plants, 15 cm in 7 days; detached pea—five experiments each of 20 tips, 47 mm in 7 days; corn—six experiments each of 10 seedlings in duplicate, 65 mm in 48 hours; and timothy—five experiments each of 20 seeds in triplicate, 8 mm in approximately 48 hours. Other details in text.

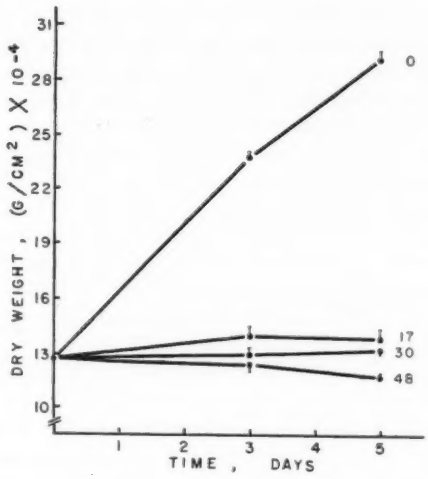


FIG. 3. Effect of 0, 17, 30, and 48 μg monuron/g fresh leaf on dry matter production of detached bean leaves. The leaves were detached Aug. 17 from 2½-week-old plants. Each value is the average for 10 leaves. Vertical lines represent one half of the standard error.

A green alga (*Chlamydomonas* sp.) growing as a contaminant in the frogbit cultures had the same dosage response curve to monuron as did frogbit roots. The algae grew profusely at 0 and 0.062 p.p.m. monuron but 0.5 and 1.0 p.p.m. severely suppressed growth. Monuron, presumably by poisoning photosynthesis in the alga, was preventing its growth. Fitzgerald (8) found that the growth of 21 different species of algae was completely inhibited by concentrations of 0.2 to 2.0 p.p.m. of monuron.

*Long-term Treatments*

The short-term application of a 50 or 100 p.p.m. solution of monuron to the petiole of a detached leaf as employed in these studies differs markedly from field treatment. Applications of 1 or 2 lb/acre as a pre-emergent herbicide would no doubt produce concentrations much less than 100 p.p.m. in the soil solution and treatment, of course, would be continuous. To check the possibility that the short-term applications might lead to erroneous conclusions, some long-term treatments of continual application of 0.1 and 1.0 p.p.m. monuron to detached leaves were carried out. For comparison short-term treatments of 5 and 10 μg monuron per gram fresh leaf were made. Typical results from these experiments are presented in Fig. 4. Other than quantitative differences, to be expected because of differences in internal concentration and in the condition of the leaves, the effect of the monuron in poisoning photosynthesis and in either enhancing or suppressing transpiration did not differ in the two types of treatments. To all appearances the short-term treatment with monuron produced typical toxic reactions in the detached leaves.

It is of interest in connection with the effect of the low doses of monuron on detached leaves that a very low dose (1–2 $\mu$g/g) of monuron stimulated both dry matter increase and rate of transpiration (day 1, 0.1 p.p.m., Fig. 4B), whereas a somewhat higher dose (5–10 $\mu$g/g) suppressed dry matter increase while stimulating transpiration (1.0 p.p.m., Fig. 4B).

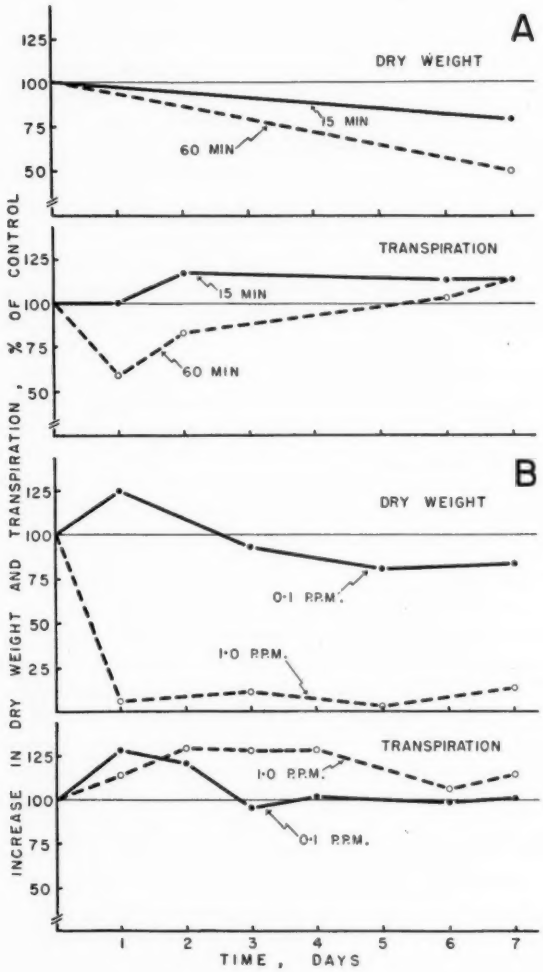


FIG. 4

*Other Inhibitors*

The effects of various respiratory and photosynthetic poisons on the physiology of detached bean leaves is given in Table IV. Internal concentrations close to or exceeding 100 μg/g fresh leaf of thiourea, sodium diethyldithiocarbamate, sodium fluoracetate, ethyl-NN-diphenylcarbamate, and hydroxylamine hydrochloride produced no symptoms and were without pronounced specific effects on photosynthesis or transpiration. 8-Hydroxyquinoline produced very slight symptoms and reduced dry matter increase somewhat but when compared with the effect of monuron a relatively high internal concentration (131 μg/g) was required to produce these effects. 2,4-Dinitrophenol, iodoacetamide, and *o*-phenanthroline all produced acute necrotic blotch-like areas which quickly turned brown and died. Because of the similarity of this blotching to one of the symptoms produced by monuron the effects of internal concentrations of approximately 20 and 40 μg/g of these chemicals were investigated in greater detail. The acute blotching from 2,4-dinitrophenol and *o*-phenanthroline treatments developed at the base of the leaf between the midrib and the main veins in comparison with the typical marginal blotches produced by monuron. This difference in the pattern of blotch development indicates a difference in mobility of these chemicals within the leaves. *o*-Phenanthroline produced extensive wilting in the leaves and, although in these particular experiments wilt was not evident from the monuron treatments, this type of symptom has been produced by monuron on other occasions (16). None of the three poisons, *o*-phenanthroline, iodoacetamide, or 2,4-dinitrophenol, produced the chronic or slow-developing types of symptoms that are typical of monuron treatments of less than 100 μg/g fresh leaf (16). As far as the action of the various poisons on photosynthesis and transpiration were concerned those of *o*-phenanthroline came closest to monuron. On the basis of similar internal concentrations, however, monuron had a more drastic effect on both photosynthesis and transpiration than did *o*-phenanthroline.

When the two closely related chemicals 3-phenyl-1,1-dimethylurea (fenuron) and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) were compared with

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FIG. 4. Effect of a low dose of monuron on dry weight increase and transpiration of detached bean leaves. All points are average values given as percentage of water controls.

FIG. 4A. Short-term treatment of 128 p.p.m. monuron for 15 and 60 minutes to give average internal concentrations of 5 and 10 μg/g fresh weight respectively. Each value is the average for 10 leaves. Leaves detached Jan. 11 from 4½-week-old plants. Mean dry weight at time of detachment was .00193 ± .000044 g/sq. cm. Average increase in weight of water control leaves was .00311 g/sq. cm. Average rate of transpiration of water control leaves in mg/sq. cm × hr was 5.6, 3.9, 3.2, and 2.3 for 1, 2, 6, and 7 days respectively.

FIG. 4B. Long-term treatment of continual application of 0.1 and 1.0 p.p.m. monuron to give average internal concentrations at the end of the first day of 0.7 and 7 μg/g fresh weight respectively. Internal concentrations on subsequent days not calculated because of the possibility of selective uptake. Leaves detached May 1 from 4-week-old plants. Mean dry weight at time of detachment was .003 ± .00005 g/sq. cm. Average increase in weight of water control leaves was .00087, .00132, .00195, and .00188 g/sq. cm for 1, 3, 5, and 7 days respectively. Each value is the average for eight leaves. Average rate of transpiration of water control leaves in mg/sq. cm × hr and the number of leaves in each group was 8.7, 28; 4.3, 22; 3.8, 22; 3.3, 15; 3.4, 7; and 2.9, 7 for 1, 2, 3, 4, 6, and 7 days respectively.

TABLE IV

Effect of poisons on symptom development, dry weight increase, and transpiration of leaves detached from bean plants grown in the greenhouse in the months of September, October, and November

| Chemical | Number leaves | Av. internal concentration, μg/g fresh weight | Av. leaf area affected, % | | | Av. change in dry weight | | | Transpiration | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | First day after treatment | | Third day after treatment | |
| | | | Acute blotch | Chronic blotch | Wilt | Duration of test, days | Change in weight, mg/cm² | % of water control | Rate, mg/cm²×hr | % of water control | Rate, mg/cm²×hr | % of water control |
| 3-(4-chlorophenyl)-1,1-dimethylurea (monuron) | 30 | 22 | 15 | 4 | 0 | 5 | + .13 | 6 | 5.0 | 56 | 4.2 | 111 |
| | 30 | 43 | 36 | 19 | 0 | 5 | − .11 | 0 | 3.8 | 42 | 2.9 | 76 |
| *o*-Phenanthroline | 10 | 24 | 50 | 0 | 60 | 5 | + .94 | 42 | 6.9 | 75 | 2.5 | 68 |
| | 10 | 44 | 70 | 0 | 70 | 5 | + .71 | 32 | 6.6 | 72 | 2.5 | 68 |
| Iodoacetamide | 10 | 25 | 0 | 0 | 0 | 5 | +1.55 | 74 | 11.2 | 127 | 2.9 | 76 |
| | 10 | 57 | 10 | 0 | 0 | 5 | +1.20 | 57 | 10.6 | 120 | 2.9 | 76 |
| 2,4-Dinitrophenol | 10 | 21 | 0 | 0 | 0 | 5 | +2.15 | 119 | 7.1 | 79 | 5.2 | 139 |
| | 10 | 42 | 17 | 0 | 0 | 5 | +1.30 | 72 | 3.2 | 36 | 4.7 | 127 |
| 8-Hydroxyquinoline | 5 | 131 | 0 | 2 | 50 | 3 | + .79 | 61 | 7.4* | 97 | 2.8 | 78 |
| Thiourea | 5 | 150 | 0 | 0 | 0 | 3 | + .95 | 73 | 8.1 | 107 | 4.1 | 114 |
| Sodium diethyldithiocarbamate | 5 | 133 | 0 | 0 | 0 | 3 | +1.04 | 80 | 4.4* | 58 | 4.1 | 114 |
| Sodium fluoracetate | 5 | 126 | 0 | 0 | 0 | 3 | +1.15 | 86 | 6.3* | 95 | 3.7 | 97 |
| Ethyl-NN-diphenylcarbamate | 5 | 83 | 0 | 0 | 0 | 3 | +1.14 | 86 | 4.3* | 65 | 4.5 | 118 |
| Hydroxylamine hydrochloride | 5 | 124 | 0 | 0 | 0 | 3 | +1.30 | 100 | 12.7 | 167 | 3.8 | 106 |

*Wilting occurred in both water controls and treated leaves during the early stages of the experiment with these chemicals.

monuron in detached bean leaves all three chemicals produced the same types of acute and chronic necrotic blotches, a similar suppression of dry matter production, and the same pattern of effect on transpiration.

*Stomatal Aperture*

Information regarding stomatal aperture can be obtained from the lack of effect of monuron on wilting. Extensive wilting developed in 9 of the 50 experiments and no wilting in 10 experiments. As mentioned in the Methods section, monuron treatment neither increased nor decreased the number of leaves that wilted. If monuron treatment were to consistently reduce stomatal aperture in a direct manner either by preventing stomatal opening at the beginning of a light period or by promoting stomatal closure after opening had occurred, then one would anticipate at least a slowing down of the wilting process. The absence of any effect on wilting suggests that monuron was not affecting stomatal aperture in a direct manner.

In the present studies where monuron produced a partial suppression of transpiration, the control leaves held on water also exhibited with time a gradual falling off of transpiration (Fig. 5). The work of Loftfield (11), who claimed that in detached shoots stomatal movement and transpiration parallelled each other quite closely and, furthermore, that the stomatal movement in detached shoots differed markedly from the movement in intact plants, suggests the possibility that the reductions in rate of transpiration found in the present studies might be related to effects on stomatal aperture.

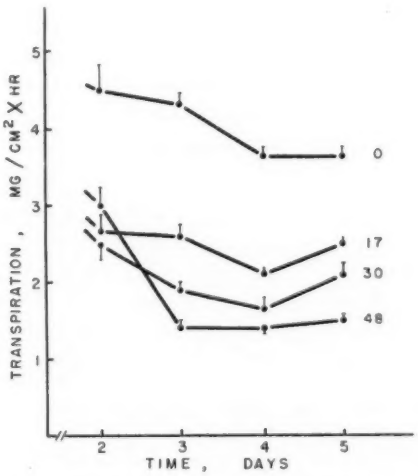


FIG. 5. Effect of 0, 17, 30, and 48 μg monuron/g fresh leaf on transpiration of detached bean leaves. The water loss was determined for each 24-hour period and the rate of transpiration calculated per hour of the 16-hour light period. These are the same leaves for which dry weights are given in Fig. 3. Each value for days 2 and 3 is the average for 20 leaves and that for days 4 and 5 is the average for 10 leaves. Vertical lines represent one half of the standard error.

In separate experiments where relative humidity and light intensity were manipulated in controlled environmental rooms the rate of transpiration of detached primary leaves of bean was varied from 10 to 19 mg of water per sq. cm of leaf per hour. None of these leaves wilted at any time. It was found that whatever the cause of the gradual falling off of the rate with time, the transpiration of detached leaves on water responded to changes in the environment in a normal and expected manner.

Although a valid theory to explain stomatal movement has not been accepted by all workers, exact knowledge is available on the effect of light, $CO_2$, and water content on stomata. Ketellapper (10) in a recent review concluded that $CO_2$ concentration occupies a very central position in the regulation of stomatal movement. One of the main types of light action is considered to be an indirect effect wherein photosynthesis reduces the $CO_2$ content within the leaf and thereby produces stomatal opening. It follows, therefore, that a poison which suppresses photosynthesis would affect the $CO_2$ concentration which in turn could affect stomatal aperture. In the present studies the leaves were transferred from 50 to 1200 ft-c of light coincident with the start of monuron treatment and depressed transpiration rates were evident in treatment periods of 1 hour's duration. The stomata were opening in the early part of the treatment period, presumably because of a reduced $CO_2$ content mediated by photosynthesis, but poisoning of photosynthesis by monuron would stop this process. Thus even if monuron treatment were found to affect stomatal aperture the effect could be an indirect one mediated by the poisoning of photosynthesis in guard cells and in the mesophyll. The difficulty in separating cause and effect, especially when the effect in all probability is indirect, made it appear pointless to determine the effect of monuron treatment on stomatal aperture at this stage of the investigation.

## Discussion

The marked inhibition by monuron of carbohydrate production in detached leaves of bean and of extension growth in the chlorophyll-containing roots of frogbit is believed to indicate that this chemical poisons the photosynthetic process. It is further believed that this poisoning of photosynthesis is related directly to certain of the slow-developing phytotoxic effects of this chemical.

The importance of light in the development of the toxic effect (14) suggests that monuron is active in the photochemical or water photolysis phase of photosynthesis, but the results from the present study do not indicate which phase of photosynthesis is poisoned or for that matter do they indicate if monuron is acting generally as a narcotic or specifically as a poison for a catalyst. Wessels and van de Veen (18) and Cooke (6) reported that monuron is a potent inhibitor of the "Hill Reaction". This reaction is believed to represent the photochemical phase of photosynthesis as it shares with photosynthesis in the conversion of light energy into chemical energy and the appearance of molecular oxygen. Bishop (1) found that the closely related substituted urea diuron reacts specifically with some intermediate chemical or enzyme

concerned with the production of oxygen in photosynthesis and has pointed out that in this reaction its resembles the effects reported by Gaffron (9) for *o*-phenanthroline and hydroxylamine. The results from the present studies with detached leaves indicate that except for differences in critical concentration levels the physiological effects of diuron, monuron, fenuron, and *o*-phenanthroline are similar. Hydroxylamine was not active in detached leaves but it may not have been reaching the required site. All evidence obtained to date, therefore, supports the suggestion that monuron inhibits the primary photochemical phase of photosynthesis and in particular that part of the phase involving the photolysis or light-induced splitting of water.

This poisoning of photosynthesis with the resultant deprivation of food and energy in all probability was the factor responsible for the conclusion of Christoph and Fisk (5) that meristems of shoots and roots were sites of monuron concentration and important loci of response to the herbicide.

If, as suggested above, monuron suppresses the photolysis of water in the photosynthetic reaction, is this suppression responsible for the 40 to 50% reduction noted in transpiration? Quantitatively only 0.6% of the transpiration water is required to account directly for the increase in dry weight of the water control leaves in Fig. 3. Moreover, internal concentrations of monuron in excess of 15 $\mu$g/g produced a simultaneous suppression of both dry matter production and transpiration and concentrations of 1 or 2 $\mu$g/g produced simultaneous enhancement of these processes, but intermediate doses of 5 to 10 $\mu$g/g suppressed dry matter production while increasing transpiration. It is doubtful, therefore, if the effects on transpiration can be related directly to the action of monuron on photosynthesis.

Present-day concepts tend to the belief that the mechanism of photosynthesis reacts through a cyclic process or processes as a result of which such diverse substances as carbohydrates, proteins, and acids arise simultaneously by photosynthetic action. It may be that the action of monuron on such a photosynthetic mechanism explains its effect on nitrogen metabolism and on transpiration as noted in the present paper. Certainly the results from the present work can more easily be interpreted as supporting the growing body of information that a fair proportion of the water passing through a plant is involved in physiological processes some at least of which are associated with photosynthesis, rather than supporting the impression given in most text books that transpiration is basically a process of doubtful value to which the plants must accommodate themselves in order to achieve gaseous exchange.

## Acknowledgments

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# OCCURRENCE OF LIGNICOLOUS FUNGI IN NORTHERN ATLANTIC AND PACIFIC MARINE LOCALITIES[1]

S. P. Meyers and E. S. Reynolds

## Abstract

The submergence and systematic examination of basswood and yellow pine panels at the far northern marine sites at Kodiak, Alaska; Nanaimo, British Columbia; St. Andrews, New Brunswick; Halifax, Nova Scotia; Liverpool, Nova Scotia; and Argentia, Newfoundland, during periods of water temperature of 0° C to 15° C, has demonstrated the abundance of an active ascomycetous and deuteromycetous mycota. The dominant fungal biota includes *Lulworthia*, *Ceriosporopsis*, *Peritrichospora*, and *Halosphaeriopsis*, and the Imperfect genera *Piricauda*, *Humicola*, and representatives of Helicosporae. Incubation of these infested wood panels has indicated fungal attack upon the panels in addition to that found at the initial examination, and has facilitated a more complete evaluation of the total lignicolous mycota, as well as establishing the early infestation of the wood. Differences in the dominant mycota between northern and southern environments are apparent, especially among the Deuteromycetes. Various genera and species extremely prevalent in warmer oceanic areas have not been found in colder marine environments.

## Introduction

Within the past decade considerable information has accumulated on the presence and relative abundance of lignicolous marine-occurring species of Ascomycetes and Deuteromycetes (7). Investigators in marine localities throughout the world have observed the occurrence of various representatives of this fungal biota. In 1957, studies were started at this laboratory on the wood-inhabiting fungal population in northern marine localities, especially in those areas where the monthly mean water temperature is less than 15° C, and often below 10° C. Other than the pioneering efforts of Barghoorn and Linder (1) in New England, and the work of Höhnk (4, 5) in the North Sea, the lignicolous fungal biota of northern marine areas had been neglected. Information on this mycota, including data on seasonal occurrence, is a necessary prerequisite to more extensive studies of the dynamics of fungal activity in the sea.

The current work reports the nature and intensity of fungal attack on basswood and yellow pine panels, submerged over various periods of time, including fall and winter months, at six northern sites: Kodiak, Alaska; Nanaimo, British Columbia; St. Andrews, New Brunswick; Halifax, Nova Scotia; Liverpool, Nova Scotia; and Argentia, Newfoundland. Water temperatures, as recorded by the correspondents, are given for each test to permit a preliminary evaluation of the effect of this factor on physiological activity of the fungi collected. The Helicosporae representatives are included provisionally in the taxon *Helicoma* (sensu Linder, 1944).

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Contribution No. 252 from The Marine Laboratory, University of Miami, Miami, Florida. These studies were supported by the Microbiology Branch, Office of Naval Research, ONR Project No. 103-305.

## Materials and Methods

Basswood (*Tilia americana*) and southern yellow pine (*Pinus palustris*) panels, approximately $2\times5\times\frac{1}{4}$–$\frac{1}{2}$ in. arranged in a "sandwich-like" manner, were used (12). This arrangement of the wood panels allowed the exposure of a maximum number of surfaces for fungal colonization and sporulation with minimum destruction and erosion of the wood by fouling organisms and by the abrasive action of sea water and of detritus. The four to six panels of each set were collected as a unit and examined individually, thus permitting an estimate of variability in sporulation between the pieces of wood.

The complete procedure of collection and shipment of exposed wood to this laboratory was described in an earlier paper (12). The scientific and naval personnel co-operating in this program were provided with a list of instructions to facilitate the removal of the panels from the exposure sites as requested. The transit period, with the wood contained within special waterproof bags, was from a few days to 1 week, depending on the particular locality.

Following receipt of the wood at The Marine Laboratory, an initial examination was made to tabulate the types and amount of fungi present and to transfer spores and mycelial growth to nutrient media for later cultural studies. Each panel was given a thorough examination since the number and concentration of fungal genera and species varied among wood pieces of individual sets. Many of the panels, especially basswood, that showed only mycelial growth initially subsequently developed fructifications of various Ascomycetes and Deuteromycetes, after a period of incubation to establish the *total* lignicolous flora present (12). The length of incubation was variable

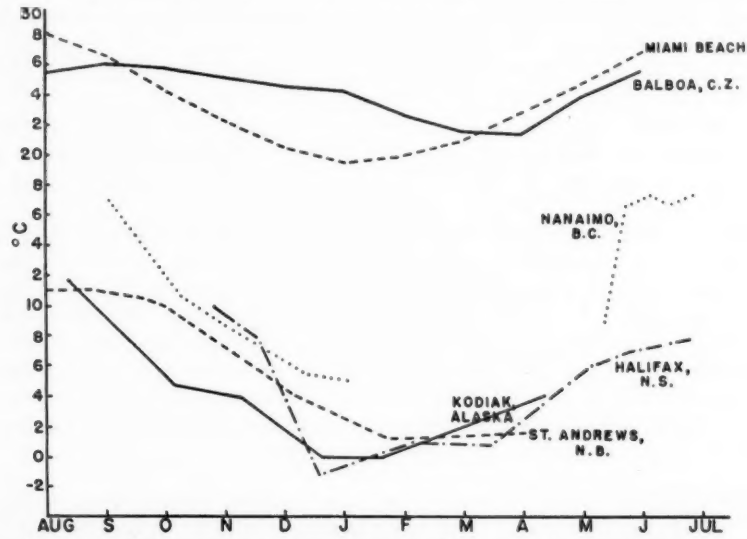


FIG. 1. Water temperatures at northern and southern marine localities during submergence period.

depending on the initial condition of the wood and on the rate at which additional fungal growth developed. The average effective incubation period was usually 3 to 4 weeks, during which time several microscopic examinations of the wood were made.

The following sections, with included tables and figure, contain the experimental data indicating the amount of fungal infestation, both initial and that developing during incubation. The length of each exposure and the temperature of the water at the time of submergence and collection, and other pertinent remarks, are included. Figure 1 shows the water temperatures at four of the northern localities during the tests. Data for Nanaimo, B.C., includes submergences during separate seasons. For comparative purposes, the monthly mean water temperatures at Miami Beach, Florida, and Balboa, Canal Zone (13), are graphed in the upper portion of the figure.

*Kodiak, Alaska*

Basswood and yellow pine panels were submerged from August 9, 1957, to April 8, 1958, at the extreme outer end of Permanent Pier (Tanker and Tender Pier), Womens Bay, Kodiak Naval Station. The water temperature at the beginning of the test was 14° C.

Fungal infestation of basswood, submerged for 242 days, is summarized in Table I. The effectiveness of the incubation process in enhancing further development from incipient fungal growth is striking, especially in the 88-day test. The extent of mycelial growth on the latter wood at the initial examination was considerably greater than that present initially on the 52-day wood. It is noteworthy that extensive development of perithecia of *Lulworthia* and *Ceriosporopsis* occurred on basswood under natural conditions when water temperature was below 5° C. Vigorous degradation of the outer wood tissues occurred on the 129-day material.

TABLE I

Infestation of basswood at Kodiak, Alaska

| Total period of exposure, days | Water temp., ° C | | Fungi present | |
|---|---|---|---|---|
| | Submergence | Withdrawal | Initial examination | Incubation period |
| | 14 | | | |
| 52 | | 7 | Mycelial growth | *Piricauda*<br>*Helicoma* |
| 88 | | 6 | Mycelial growth | *Helicoma*<br>*Piricauda*<br>**Humicola*<br>*Halosphaeriopsis* |
| 129 | | 2 | **Lulworthia*<br>*Ceriosporopsis* | — |
| 159 | | 2 | *Lulworthia* | **Piricauda*<br>*Humicola*<br>*Halosphaeriopsis*<br>*Peritrichospora* |
| 242 | | 6 | *Lulworthia* | *Piricauda*<br>*Humicola*<br>*Peritrichospora* |

*Dense colonization over entire wood surface.
— No incubation period.

Development and reproduction by *Lulworthia*, *Ceriosporopsis*, and *Halosphaeriopsis*, although vigorous on the basswood, was not observed on the yellow pine panels. The earliest evident fungal sporulation on pine was that of *Helicoma* at the initial examination of the 52-day panels. Conidia of *Piricauda* developed on this same wood during the incubation process. Throughout the total exposure period of 242 days, only *Helicoma* was observed on the pine panels initially. However, sporulation by *Piricauda* and *Humicola* occurred on these panels during incubation. The sole ascomycetous fungus noted on pine was *Peritrichospora*, developing during incubation of the 159-day wood.

*Halifax, Nova Scotia*

Basswood panels were submerged from October 23, 1957, to May 22, 1958, from a wooden jetty at the entrance to Halifax Harbor. The water temperature at the beginning of the test was 12° C.

Initial examination of different sets of panels, submerged for periods of 21, 56, 92, and 138 days, revealed only increasing intensities of mycelial development (Table II). However, sporulation of species of *Ceriosporopsis*, *Peritrichospora*, *Piricauda*, and *Humicola* occurred during the incubation of the 56-, 92-, and 138-day wood. It is interesting that the fructifications of *Ceriosporopsis*, *Lulworthia*, and *Piricauda*, not present initially until after 191 days, were produced during the incubation of the 56-day wood, thus demonstrating the early infestation relative to subsequent sporulation.

TABLE II

Infestation of basswood at Halifax, Nova Scotia

| Total period of exposure, days | Water temp., ° C | | Fungi present | |
|---|---|---|---|---|
| | Submergence | Withdrawal | Initial examination | Incubation period |
| | 12 | | | |
| 21 | | 10 | Mycelial growth | — |
| 56 | | 1 | Mycelial growth | *Ceriosporopsis*<br>**Peritrichospora*<br>*Piricauda*<br>*Humicola* |
| 92 | | 3 | Mycelial growth | **Ceriosporopsis*<br>*Peritrichospora*<br>*Piricauda*<br>*Humicola* |
| 138 | | 3 | Mycelial growth | **Ceriosporopsis*<br>*Peritrichospora*<br>*Lulworthia*<br>*Piricauda* |
| 191 | | 8 | **Lulworthia*<br>*Ceriosporopsis*<br>*Piricauda* | — |
| 201 | | 6 | **Lulworthia*<br>**Ceriosporopsis*<br>*Piricauda*<br>*Helicoma* | — |
| 212 | | 9 | **Lulworthia*<br>**Ceriosporopsis*<br>*Piricauda*<br>*Speira* | *Peritrichospora* |

*Dense colonization over entire wood surface.
— No incubation period.

The wood submerged for 21, 56, and 92 days showed only slight development of fouling organisms, with no activity by *Limnoria* or *Teredo*. Panels with a submergence period of 138 days, or longer, and carrying over into the spring months, exhibited extensive attack by these wood-boring organisms.

*St. Andrews, New Brunswick*

Basswood and yellow pine panels were submerged from July 29, 1957, to May 26, 1958, at the wharf of the Biological Station. The water temperature at the beginning of the test was 12.5° C.

Infestation of basswood and yellow pine at this locality is summarized in Table III. Basswood, submerged for 300 days and longer, exhibited dense colonization by *Lulworthia* and *Ceriosporopsis*, with mature perithecia of these genera over all wood surfaces. Neither of these fungi was observed earlier than 300 days. At Halifax, Nova Scotia, and Kodiak, Alaska, basswood submerged for 138 and 129 days respectively, with the water temperature less than 5° C, showed vigorous sporulation by *Lulworthia* and *Ceriosporopsis*.

TABLE III

Infestation of basswood and yellow pine at St. Andrews, New Brunswick

| Total period of exposure, days | Water temp., ° C | | Wood | Fungi present | |
|---|---|---|---|---|---|
| | Submergence | Withdrawal | | Initial examination | Incubation period |
| | 12.5 | | | | |
| 22 | | 14 | Basswood | Mycelial growth | * |
| | | | Pine | None | * |
| 49 | | 13 | Basswood | Mycelial growth | *Graphium* |
| | | | Pine | *Helicoma* | * |
| 78 | | 11 | Basswood | *Piricauda* | * |
| | | | Pine | *Helicoma* | * |
| 126 | | 6 | Basswood | Mycelial growth | *Piricauda*<br>*Humicola*<br>*Peritrichospora*<br>*Halosphaeriopsis* |
| | | | Pine | *Helicoma*<br>*Leptosphaeria* | — |
| 176 | | 2.5 | Basswood | † | *Piricauda*<br>*Humicola*<br>*Peritrichospora*<br>*Halosphaeriopsis* |
| | | | Pine | † | *Helicoma*<br>*Humicola* |
| 300 | | 4 | Basswood | *Lulworthia*<br>*Ceriosporopsis* | — |
| | | | Pine | *Helicoma* | — |

*No further types of fungal sporulation.
†No initial examination of wood.
— No incubation period.

*Nanaimo, British Columbia*

Basswood and yellow pine panels were submerged from September 4, 1957, to January 1, 1958, ¼ mile out in Departure Bay opposite the Biological Station. The water temperature at the beginning of the test was 19° C.

Within 37 days, the basswood showed extensive colonization by *Piricauda* and *Lulworthia*, with *Peritrichospora* developing during incubation. This latter fungus was not observed on wood, initially, until the 96-day examination. At this time and 23 days later, large numbers of perithecia of *Peritrichospora* were present over the wood surfaces. Yellow pine showed infestation by *Helicoma* throughout the total submergence period of 119 days. Fructifications of *Lulworthia* developed on pine during incubation. Borer attack was evident on these panels at 63 days (water temperature, 10.5° C), with vigorous attack on the 119-day panels when the water temperature was 7° C.

Another series of basswood and pine panels was submerged during a later period, May 13 to June 26, 1958, at the Biological Station dock. The water temperature at the beginning of this test was 19° C. During this spring period, the extent of total fungal attack on both woods was considerably less than that observed on wood submerged for 37 days during the early fall period. The former fungal flora included species of *Helicoma*, *Piricauda*, *Alternaria*, and *Peritrichospora*. The reproductive structures of the latter fungus developed on the wood during incubation. *Alternaria* was not found on the wood submerged in the first series of tests and has not been collected at the other exposure sites. Throughout our studies, in both northern and tropical environments, this genus has been isolated infrequently in contrast to the abundance of the organism in terrestrial environments.

*Argentia, Newfoundland*

Basswood and yellow pine panels were submerged from August 1, 1957, to May 8, 1958, at the U.S. Naval Station. The water temperature at the beginning of the test was not recorded.

At 43 days, only slight mycelial growth was present on the basswood and none on the pine. No further fungal development or recognizable fructifications were noted on the wood even after incubation for 8 weeks. Basswood submerged for 121 and 183 days, with the water temperature less than 10° C, and as low as 0° C, exhibited vigorous mycelial growth. Subsequent incubation of this fungal-infested wood showed sporulation by various Ascomycetes and Deuteromycetes, including *Lulworthia*, *Ceriosporopsis*, *Peritrichospora*, *Piricauda*, and *Humicola*. During the same exposure period, *Helicoma* was the only fungus observed on the pine panels.

*Liverpool, Nova Scotia*

Basswood and yellow pine panels were submerged from August 15, 1957, to February 4, 1958, at the facilities of the Mersey Paper Co. Ltd., where the water temperature at the beginning of the test was 17° C.

After 62 days, fungal infestation was not observed on basswood, but *Helicoma* was abundant on pine both in this test and throughout the entire exposure

period. Further examinations of basswood exposed for 113 and 173 days, with the water temperature at 1° C, showed vigorous mycelial growth. Perithecia of *Lulworthia* were noted on the 173-day panels. *Piricauda* developed on the latter wood during incubation. The only ascomycete observed on pine was *Leptosphaeria*, noted after 173 days' submergence.

## Discussion

Little direct information is available on the effect of water temperature on the development of lignicolous marine fungi in their natural habitats. Earlier workers (1), drawing upon records of collections made during two different times of the year, observed that differences in water temperature seemed to affect the formation of the sexual stages in certain species of *Lulworthia*. However, terminal temperature data alone are rather inconclusive regarding their effect on the maturation of fungal reproductive structures on submerged wood. An accurate study of the relation of fungal occurrence and abundance, including reproduction, to water temperature requires an analysis of seasonal mean temperatures together with the pattern of successive fungal infestation of the wood.

Barghoorn and Linder (1) conducted laboratory experiments designed to evaluate the reactions of selected species to various temperatures as indicated by their rate of radial mycelial growth in culture. Their data showed a requirement by these organisms for a relatively high temperature for optimal radial growth. Only three species had an optimal growth temperature below 25° C. However, all of the species grew to an "appreciable extent" at 5° C, and in one species of *Lulworthia* the growth rate was relatively high at this temperature. Cultures maintained between 0° C and 1° C showed some slight but nevertheless appreciable growth. Barghoorn concluded, "From the available data it is evident that the marine fungi, despite relatively high temperature optima, are capable of growth and development at temperatures no higher than those of arctic oceanic waters." The data given in the present paper readily support these latter conclusions.

It is recognized that the distribution and reproduction of fungi in the sea result from various complex processes in which many ecological and physiological factors participate. Rather than to attempt to characterize the role of water temperature per se in the dynamics and ecology of fungal activity, we have studied the nature and general succession of the mycota on submerged wood during periods of low water temperature. Undoubtedly, other factors such as salinity and general biologic activity influence fungal growth to a considerable extent, and recently, attention has been given to the effect of a combination of salinity and temperature on the distribution of certain lignicolous species (3).

The fungal distribution indicated here represents the dominant populations observed, using a combination of field and laboratory methods in the isolation procedure. It is quite possible that various, less abundant, species may have been overlooked in our series of examinations of individual infested panels.

However, the incorporation of an incubation procedure into our methodology has extended greatly our knowledge of both the wood infestation process and the fungi involved. Through incubation it is possible to ascertain the early or initial fungal attack upon the wood prior to development of the reproductive structures.

The ascomycetous genera *Lulworthia*, *Ceriosporopsis*, and *Peritrichospora*, occurring abundantly in these nothern sites, have been isolated from marine localities in various parts of the world. Species of these genera often comprise a large part of the fungal population on submerged wood. Frequently, especially in warmer oceanic areas, species of *Lulworthia* colonize wood within a few weeks following its submergence. The genus *Halosphaeriopsis*, common in our collections, has been reported from various localities along the U.S. Atlantic seaboard as well as from Australia (6).

Certain lignicolous genera and species collected in warmer oceanic areas have not been found in the six northern localities studied here. Of particular interest is the ascomycetous fungus *Antennospora quadricornuta* (Cribb & Cribb) Johnson, collected regularly on wood in subtropical and tropical marine environments (9, 10), including the Caribbean, Bahamas, Gulf of Mexico, and in the South Pacific at Tahiti and Australia. Recently, the presence of this species in the Indian Ocean was reported (2). Extensive distributional evidence on the occurrence of *A. quadricornuta* suggests that the species is a significant member of the lignicolous mycota of warmer marine habitats. In our previous work, the northernmost area in which this species had been isolated was Ft. Pierce, Florida, but Johnson (6) noted the presence of the fungus on submerged wood in the Beaufort Inlet region of North Carolina.

In comparing the mycotas of northern and arctic areas with those of the subtropical and tropical localities, other differences of ecological significance are evident. While the same genera, and even species, are present occasionally in more than one area, the major or *dominant fungal biotas* present do differ between localities. In view of the considerable differences between the average mean of the monthly water temperature at Miami Beach, Florida, 25.8° C, and Balboa, Canal Zone, 26.8° C, and that of Womens Bay, Kodiak, Alaska, 5.6° C (13, 14), the occurrence of dissimilarities in the dominant fungal biotas is not surprising. The following discussion relates to these latter gross differences based on a summarization of the infestation pattern observed through extensive examinations.

The abundance of species of Helicosporae in northern localities has been noted previously (11). However, the species present in northern areas generally differ from those regularly collected in southern or tropical environments. Taxonomic and physiological aspects of the helicoid complex are at present under study in this laboratory. It is interesting that *Cirrenalia macrocephala* Meyers and Moore, a species described by Kohlmeyer (8), while common in temperate and warmer oceanic areas, occurred infrequently or not at all in the localities discussed here.

In addition to species of *Helicoma*, two other deuteromycetous fungi, *Humicola alopallonella* Meyers & Moore and *Piricauda arcticoceanorum* Moore, were extremely abundant on submerged wood in northern areas. Often, representatives of the two latter genera constituted the major lignicolous fungal biota present. This study is the first report of the presence of *Humicola* in a marine environment. Earlier work in tropical and subtropical areas did not show the occurrence of the fungus on either basswood or yellow pine panels. *Piricauda articoceanorum*, collected at all of the exposure sites, is one of the earliest species to colonize submerged wood. Another species of *Piricauda*, *P. pelagica*, described by Johnson (6) from driftwood along the North Carolina coast, has been found also on pine panels at Annapolis, Maryland, and in the Panama Canal Zone. However, in general, the genus *Piricauda* is absent from warmer oceanic areas, and to date, has not been observed to be a dominant wood inhabitant in such localities.

As continued ecological investigations of marine-occurring fungi develop, it is quite likely, in view of the dissimilar mycotas on different woods, that heretofore unreported genera and species will be discovered, thus presenting valuable contributions to further our basic understanding of the dynamics of lignicolous fungal activity in diverse marine areas. To date, initial studies of the dominant wood-inhabiting fungal biota in arctic and tropical areas indicate that noteworthy differences in the characteristics of the population do occur. The significance of these differences requires further study. However, the presence of such a fungal population in arctic and northern marine areas necessitates a consideration of their diverse degradative and synthetic processes in subsequent investigations of the plant and animal life of these localities.

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# GROWTH OF THE RUST FUNGUS PUCCINIA HELIANTHI ON TISSUE CULTURES OF ITS HOST[1]

CONSTANCE NOZZOLILLO[2] AND J. H. CRAIGIE

## Abstract

Rust-infected cotyledon, hypocotyl, and stem sections of sunflower seedlings were cultured on nutrient agar media. Callus growth from cotyledon sections was negligible, but hypocotyl and stem sections proliferated more or less freely. Infections arising from basidiospores produced an abundant tuft-like growth of haploid surface mycelium. Infections arising from urediniospores lacked almost entirely surface mycelial growth, but produced urediniospores in abundance, and finally teliospores.

Copious aeciospore production occurred in some cultures where two basidiospore infections of different mating types coalesced. Later such compound infections produced urediniospores and teliospores. Tufts of mycelium developed on the new callus growth of some cultures. One of these calluses (examined cytologically) contained a number of more or less subnormal pycnia and aecia in different stages of development. Only a few sections produced callus tissue capable of surviving repeated subculturing. In the fourth set of subcultures from one such section, rust mycelium appeared as tufts on six of the calluses.

## Introduction

The development of techniques for the cultivation of plant tissues in artificial media (8, 11) made possible a new approach to the study of the obligate parasites the rust fungi. The growth of these fungi in tissue cultures of their respective hosts seemed to offer a step towards the goal of the saprophytic culture of plant rusts. Morel (7) was unable to infect healthy callus tissue in culture with either urediniospores or basidiospores of the appropriate rust fungi and suggested that tissue already infected with a rust fungus be used as source material. Hotson and Cutter (6) and Hotson (5) were able to obtain the growth of rust mycelium in the new growth of host tissue in cultures and subcultures of gall tissue of *Juniperus virginiana* infected with *Gymnosporangium juniperi-virginianae*. Hotson and Cutter reported that in a few cultures the mycelium of the fungus grew out from the host tissue and established itself on the agar medium. Cutter (2) later reported that four strains of the fungus were so derived, each being capable of subculture and of infecting the alternate host. Turel and Ledingham (10) failed to get this fungus to establish itself on artificial media, but succeeded with *Melampsora lini* in obtaining an abundant production of mycelium and urediniospores on infected cotyledons of flax in culture. Similarly, Bauch and Simon (1) obtained abundant mycelial growth of *Puccinia tartarica*, with the production of teliospores,

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[2]Present address: Microbiology Research Institute, Canada Department of Agriculture, Ottawa, Ontario.

on tissue cultures of rust-infected wild lettuce. Rossetti and Morel (9) cultured gall tissue induced by *Puccinia minutissima* and *Gymnosporangium juniperi-virginianae* in *Decodon verticillatus* and *Juniperus virginiana* respectively. Neither rust became saprophytically established on the medium. Cutter (3) recently published a detailed account of the work reported earlier (2, 6). Three more strains of the fungus were isolated from gall tissue cultures despite the infrequent and unpredictable occurrence of saprophytic growth of the rust mycelium. In each case, such growth was preceded by the disintegration of the host callus.

This paper reports the growth of *Puccinia helianthi* Schw. on cultures of rust-infected cotyledon, hypocotyl, and stem tissues of sunflower seedlings.

## Materials and Methods

Sunflower rust urediniospores were collected in the field and a fresh supply was continually provided by greenhouse culture on sunflower plants. Telial material, collected in the field, was kindly supplied by Dr. W. E. Sackston, of the Canada Agriculture Research Laboratory, Winnipeg, Manitoba. Sunflower seedlings (*Helianthus annuus* L. var. Russian Giant) were grown in pots in the greenhouse. Uredinial infections were obtained by spreading with the fingers a water suspension of urediniospores over cotyledon, hypocotyl, or stem surfaces. Basidiospore infections were obtained by exposing moistened seedlings in a damp chamber to a shower of spores from germinating teliospores suspended a short distance above them. The inoculations were usually made soon after the cotyledons had unfolded. Inoculated plants were kept in an incubation chamber for about 48 hours, and then placed on a greenhouse bench. Infections became visible as whitish specks in 5–7 days and the cotyledons were removed for culture 2–3 days later. At this age, hypocotyls and stems were usually quite succulent and tender and were not cut into sections until several days later, by which time the basidiospore infections appeared as orange swellings, and the uredinial infections as long, narrow, brown sori. Cotyledons were usually cut crosswise into 5- to 8-mm sections which were placed singly in the culture tubes. Stem sections were peeled and cut into 2-cm lengths each bearing an infection which, whenever possible, was located at the root end of the section, and this end was placed uppermost in the culture tube. To sterilize the tissue sections, Javex, a commercial preparation containing 7% available chlorine, was used, usually diluted 1:4 with distilled water. The sections were immersed in this solution, usually for 10 minutes, then washed with sterile water, trimmed to the desired size, and placed on the medium. An average of 50% of the cultures so prepared remained free from contaminants. The cultures were kept in darkness at 25° C, and were transferred at 6-week intervals.

Various media were tested—in all, upwards of 150 different combinations. The composition of those found most satisfactory is given in Table I. The media were dispensed into 16×150 mm test tubes, 10 ml per tube for cotyledon sections and 15 ml per tube for stem sections, the tubes for the cotyledon

sections being sloped. The tubes were then plugged with cotton and autoclaved 20 minutes at 10 pounds pressure. To control bacterial contaminants a sterile solution of aureomycin was added aseptically to each tube before the agar had solidified (final concentration 25 mg/l.).

TABLE I

Composition of five media used in the culture of rust-infected sunflower tissues

| Ingredient* | A† | B† | C‡ | D† | E |
|---|---|---|---|---|---|
| g/l. | | | | | |
| Agar | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| Sucrose | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |
| $Fe_2(SO_4)_3$ | 0.0025 | 0.0025 | 0.0025 | 0.0025 | 0.0025 |
| Yeast extract | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Peptone | 0.010 | — | — | — | — |
| NAA | — | $10^{-10}$ | $10^{-10}$ | $10^{-10}$ | $10^{-10}$ |
| ml/l. | | | | | |
| Coconut milk | 50.0 | — | 100.0 | 100.0 | — |
| *M* $Ca(NO_3)_2$ | — | — | 1.20 | — | 5.0 |
| *M* $KNO_3$ | — | — | 0.80 | — | — |
| *M* $NaNO_3$ | 2.80 | 2.80 | — | 5.08 | — |
| *M* $NH_4NO_3$ | — | — | — | — | 2.0 |
| *M* $MgSO_4$ | 0.80 | 0.80 | 2.92 | 1.68 | 2.0 |
| *M* $Na_2SO_4$ | — | — | 1.41 | — | — |
| *M* $NaH_2PO_4$ | 0.40 | 0.40 | 0.14 | 0.84 | — |
| *M* $KH_2PO_4$ | — | — | — | — | 2.0 |
| *M* KCl | 4.80 | 4.80 | 1.74 | 10.08 | — |
| *M* $CaCl_2$ | 0.20 | 0.20 | — | 0.44 | — |
| *M* $MgCl_2$ | 1.00 | 1.00 | — | — | — |
| Stock trace elements | 1.0§ | 1.0§ | 1.0§ | 1.0 | 1.0§ |

*Special agar: Noble, Brewer's Yeast Extract, Proteose Peptone, Difco Laboratories; naphthalene acetic acid (NAA), Eastman Kodak Co.; coconut milk obtained from nuts on local market and stored in freezer.
†Modified Heller's medium (4).
‡Modified White's medium (11).
§Hoagland's solution consisting of $ZnSO_4$ (55 mg/l.), $H_3BO_3$ (210 mg/l.), $CuSO_4.5H_2O$ (55 mg/l.), $MnCl_2.4H_2O$ (40 mg/l.) $(NH_4)_6$, $Mo_7O_{24}.4H_2O$ (20 mg/l.).

*Culture of Rust on Cotyledons*

The infections, barely visible at the time of setting up the cultures, developed normally on the cotyledon sections, basidiospore infections becoming orange in color as the pycnia developed. Within 4 weeks, some of these infections were 1 cm in diameter. On the surface of many infections, tufts of mycelium appeared in 10–14 days. Some of these tufts developed quite extensively, covering a considerable area of the infection site (Fig. 1), but each seemed to arise from a single point so that practically the whole tuft could readily be raised from the surface of the section. In a number of the cultures, aecia appeared, indicating that the infections were not of monosporous origin; and aeciospores were shed abundantly over the surface of the agar. Two or three weeks later black spots, indicative of uredinial and telial sori, were sometimes visible. Urediniospore infections began to shed viable spores within 4 days and teliospores developed in due course. Mycelium was observed very rarely on the surface of these infections and when it was, it appeared as fine strands and not as tufts. The infections themselves did not enlarge to the same extent as did basidiospore infections, reaching a diameter of only 2–3 mm (Fig. 2).

The cotyledon sections expanded normally, and remained green for several days. By the end of the first week, however, they began to turn yellow, and became completely brown in about 4 weeks. Some cultures developed callus growth on the cut edges, but it was not extensive and did not long survive. Root development from the cut edges was frequently observed.

*Culture of Rust on Hypocotyl and Stem Tissue*

The development of infected hypocotyl and stem sections in culture seemed to be determined by the extent to which the tissue had been injured during sterilization. In cases of severe injury, growth, if it occurred at all, became manifest as a callus on the root (uppermost) end, which reached maximum size in about 2 weeks, soon became brown, and never survived transfer. In such calluses, the cells were of the giant type, long in relation to their width, and loosely connected. No further development of rust mycelium and no increase in diameter of the section occurred. With less severe injury, the whole section expanded in length and diameter, the greatest increase in diameter being at the root end. Pronounced root growth usually developed at this end as well. On many of these sections, tufts of haploid mycelium appeared on the surface, a dense mat forming in 4–6 weeks (Fig. 3). As in cotyledon sections, each tuft seemed to debouch from a single fissure in the tissue (Fig. 4). Where more than one basidiospore infection was originally present, aeciospores, urediniospores, and teliospores appeared in some of the cultures. Such mycelial mats did not develop on urediniospore infections. At most only a few mycelial strands appeared on the surface of these, and abundant urediniospore production, such as occurred on cotyledon sections, was rarely observed. Although the initial development of these cultures was promising, all attempts failed to establish subcultures of their callus tissue. The tissue gradually deteriorated and was dead within 4 months.

Paraffined sections were prepared of the upper end of the stem section shown in Fig. 3. It was observed that mycelial growth was most abundant in the undifferentiated (callus) tissue, where the haploid mycelium gave rise to scattered pycnia (Fig. 5), some of which were of giant size, and to a number of dense hyphal wefts, which were not usually in the immediate vicinity of the pycnia. Except for some pycnia that probably were more or less developed before they were overgrown by callus tissue, the pycnia appeared to be 'blind', having no observable ostiole or ostiolar filaments. All of the latter type were filled with a dense mass of seemingly normal pycniospores. The hyphal wefts arose in intercellular spaces and pushed the neighboring host cells apart, occasionally surrounding and isolating one or more of them. These wefts seemed to be homologous with the protoaecia of haploid infections on a host leaf, the cells being uninucleate.

In this particular section, the mycelia happened to be of two different mating types. The cells of the intercellular hyphae continued to be uninucleate or very largely so, but in the dense hyphal wefts, aecia, which lacked definite orientation, were present in various stages of development. In the initial stage, some cells more or less central in the weft became binucleate (Fig. 6). In

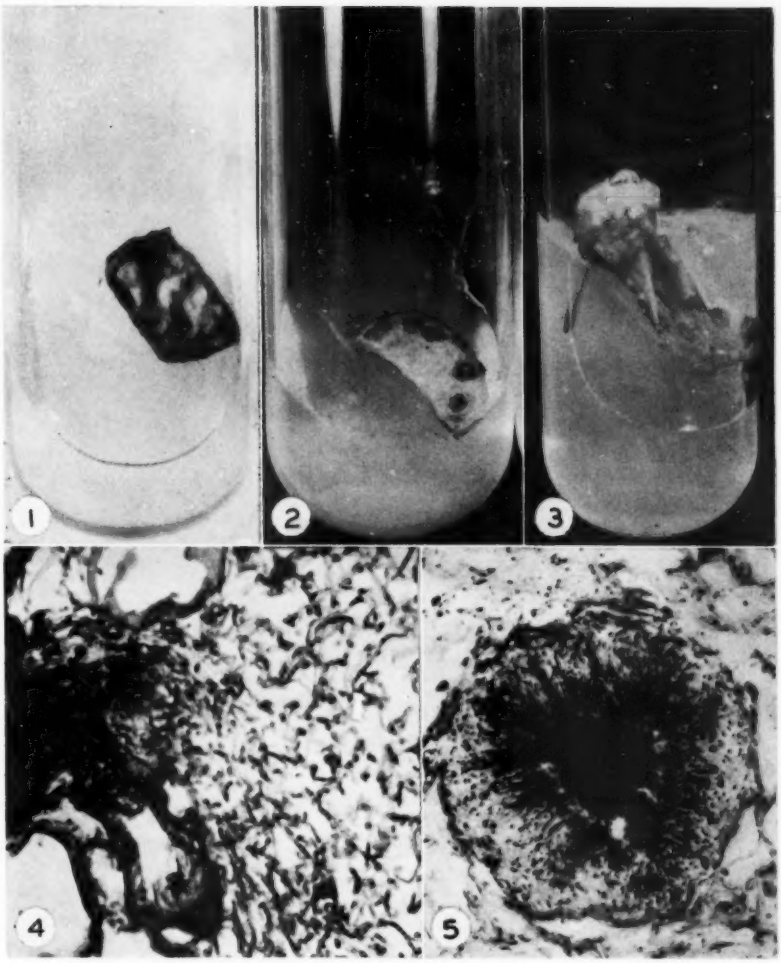



FIG. 1. Haploid mycelial growth on a cotyledon section after 3 months in culture. ×1.5

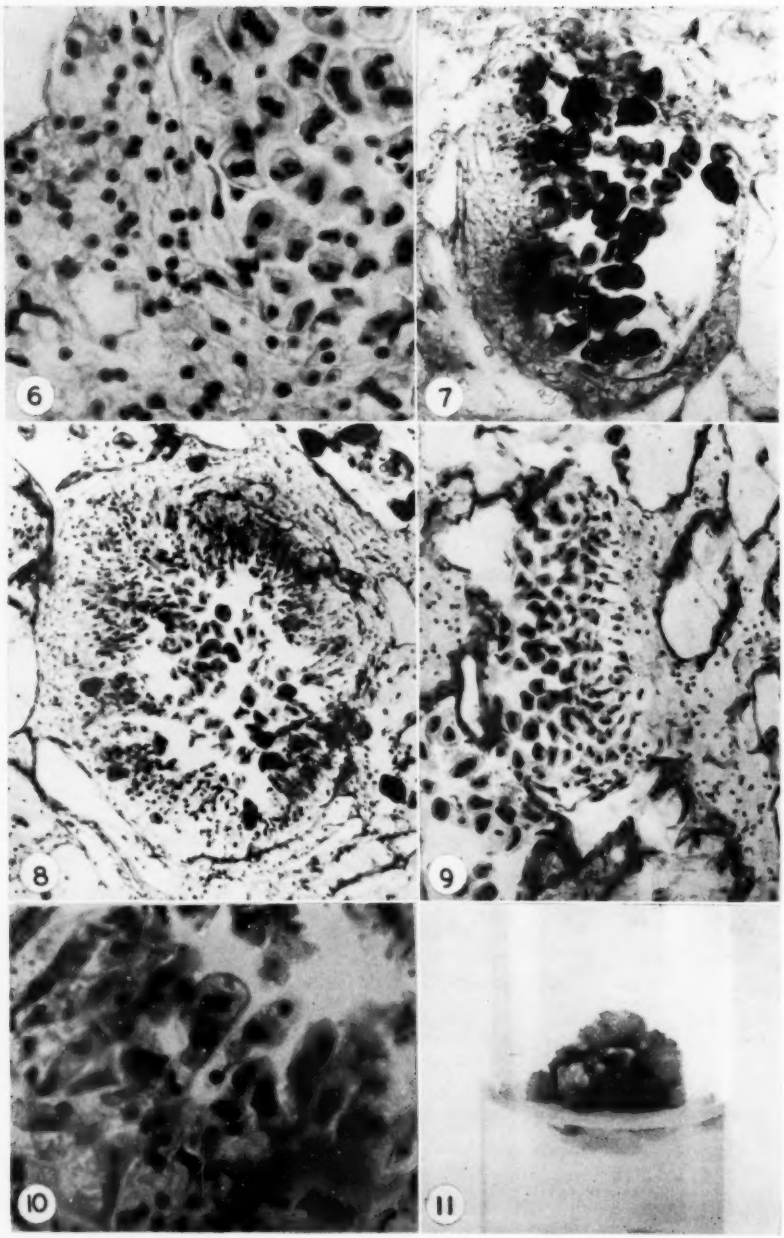
FIG. 2. Uredinial infections on a cotyledon section after 3 weeks in culture. Minute clumps of shed urediniospores lie on the agar surface. ×1.5

FIG. 3. Stem section which has expanded in length and diameter during 6 weeks in culture. A tuft of mycelium (outlined to indicate its position) is present in the center of the new white callus growth. ×1.5

FIGS 4–10. Microphotographs of sections cut through the callus tissue of the culture shown in Fig. 3. The aecia present lacked any definite orientation. All sections were stained with Giemsa solution.

FIG. 4. Section through a tuft of surface mycelium that has emerged through a fissure in the callus tissue. ×370

FIG. 5. Section through an apparently 'blind' pycnium. ×200

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the subsequent development of an aecium, only a part of the hyphal weft concerned became involved; the rest of the cells in the weft remained largely uninucleate. All the aecia were embedded in the callus tissue and were to a greater or lesser extent subnormal. They tended to be globular in shape and surprisingly large, some having a diameter double that of a normal aecium of this rust. A true peridium seemed generally to be lacking, the aecium being delimited by the surrounding cells, pressed perhaps more tightly together by the enlarging aecium. In a few aecia, the internal organization seemed more or less that of a normal aecium (Fig. 7); in a few others, the spore-producing layer occupied all or practically all of the inner periphery (Fig. 8). The latter type of aecium appeared to be 'blind', having no evident way of discharging its spores—except by disintegration of the spore-producing layer. The aeciospores of some of the aecia, especially in the younger ones, were binucleate and seemingly quite normal; but, in others, especially the older and larger aecia, they were largely abnormal. In some of these aecia, teliospores as well as aeciospores were present (Figs. 9 and 10) and in some cases both types of spores were abortive, lacking nuclei and other cell contents. Aeciospore production, even in the more normal type of aecium, was rather limited, amounting usually to not more than three or four spores in any aeciospore chain.

A small number of cultures suffered only slight sterilization damage. In such cultures, the whole tissue expanded to form a large cylinder with numerous roots growing from the uppermost end. In some of these, the rust mycelium appeared as small tufts on the surface of the enlarged tissue, but, in most, no surface mycelium appeared at all. After 6–12 weeks in culture, the tissue was too large to be transferred in one piece, and it was cut into quarters before being subcultured. In another 6 weeks, the quarter sections had proliferated to such an extent that they were again ready for subculturing, and thus a continuing growth of callus tissue was obtained. Although most of these cultures had already been infected with rust mycelium, usually of basidiospore origin, only one gave rise to callus tissue on which surface tufts of rust mycelium were visible. It is quite possible that mycelium may have been present in at least some of the others.

*Rust Growth on Callus Tissue*

The stem section from which the rust-infected callus tissue just mentioned was derived had been placed in culture with a haploid infection at the uppermost end. The section expanded more or less uniformly in diameter. In 6 weeks,

FIG. 6. Section through a haploid hyphal weft in which an aecium is already in the process of development. ×700

FIG. 7. Section through a subnormal aecium which produced some more or less normal teliospores and abortive aeciospores. ×200

FIG. 8. Section through a young apparently 'blind' aecium in which the spore-bearing area embraces the whole inner periphery. ×175

FIG. 9. Section through an aecium which lacks a peridium but is otherwise more or less normal. ×200

FIG. 10. Portion of a section through a young aecium showing an immature teliospore that is developing in the spore-producing area of the aecium. ×750

FIG. 11. A rust-infected callus tissue derived, through four successive subcultures, from a rust-infected stem section placed in culture 21 weeks previously. ×1.5

rust mycelium appeared on the surface as a small white tuft. This small growth was soon engulfed by the proliferating host tissue, which was subsequently quartered and subcultured, as described just above. After 21 weeks, a tuft of rust mycelium became visible on one of the calluses and a week later on each of two others. Figure 11 shows one of these calluses with mycelial tufts clearly visible in the center. The three calluses were divided at 24 weeks to form seven subcultures, on six of which surface rust mycelium appeared in 3 weeks. Further subculturing yielded no more calluses bearing visible rust mycelium. The vitality of the host tissue itself, even of those calluses on which no rust mycelium was visible, began to decline and the cultures gradually died out.

*Attempted Inoculation of Healthy Callus Tissue*

Various means were used in attempts to inoculate uninfected callus tissues. An abundant supply of germinable urediniospores, free of contaminants, was obtained from cotyledon cultures, and inoculations with these were made (1) by placing a spore suspension on the surface of the callus, (2) by vacuum infiltration of a spore suspension, and (3) by injection with a hypodermic needle of a spore suspension into the callus. In other attempts, pieces of cotyledon or stem sections on which there was an abundant external growth of haploid mycelium were placed in close contact with the uninfected calluses in the hope that the mycelium might invade the callus tissue. None of these inoculations resulted in visible rust infections.

*Effect of Culture Media*

In the cotyledon cultures, the type of medium did not have any visible effect, the same pattern of development occurring on all the media tested, of which medium E (Table I) was the most commonly employed. Sucrose seemed to be the only essential ingredient for urediniospore production. In hypocotyl and stem cultures, medium A (Table I) was more favorable than any of the others for initiation of proliferation of the host tissue, although subsequent callus growth seemed to occur equally well on media C and D. Growth on medium B, which lacked coconut milk, was not so rapid as in the latter two media. The decline in vigor of all callus growths, both infected and non-infected, which occurred when most of the cultures were 6–10 months old must be attributed to some unknown factor in the environment common to all the cultures. In none of the cultures was there any tendency for the rust mycelium to become established on any of the media. When the host tissue died, the rust mycelium died also.

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# FORMATION OF MICROENDOSPORES IN CERATOCYSTIS ULMI (BUISM.) C. MOREAU[1]

G. B. Ouellette and C. Gagnon[2]

## Abstract

A morphological study of several isolates of *Ceratocystis ulmi* has revealed that spores and hyphae of this fungus contain minute cytoplasmic particles that behave like spores (microendospores). The different stages of development of these microendospores are described and some implications of the findings are briefly discussed.

## Introduction

In her original account of *Graphium ulmi*, the imperfect stage of *Ceratocystis ulmi*, Schwarz (6) described three types of spores: coremiospores, conidia which originate singly or in Cephalosporium-like slimy heads on branched conidiophores, and spores that bud in a yeast-like manner. Several workers (2, 3, 9, 10) have subsequently confirmed most of Schwarz's observations, but further information on the morphology of the imperfect stage of this fungus has been scanty.

Recently, during studies on the Dutch elm disease, minute bodies which behaved like spores were observed in typical spores and hyphae of the causal fungus, *Ceratocystis ulmi*. Since these minute bodies which appeared to represent a type of endospore might play an important part in the infection mechanism, an attempt was made to study their development. This paper reports on these preliminary studies.

## Materials and Methods

The cultures examined during this study are listed in Table I. These were maintained on slants of 2% potato dextrose agar (PDA) and kept at approximately 4.5° C. Monoconidial cultures of some of the isolates were obtained by isolating single spores from a dilution plated on agar in petri dishes.

Cultures were grown on slides prepared with 2% water agar or PDA according to the method of Riddell (5), and in 125-ml flasks containing 25 ml of liquid medium sterilized at 15 lb for 15 minutes. The liquid medium contained (per liter): 25 g technical glucose, 2 g asparagine, 2 g yeast extract, 1.5 g $KH_2PO_4$, 1 g $MgSO_4.7H_2O$, 0.01 g $FeCl_3$.

The inoculum consisted of a spore suspension taken from slants or liquid cultures diluted in distilled water to a few spores per loopful. Inoculum was also prepared from liquid cultures after centrifuging the suspension at approximately 1500 *g* for 50 minutes to separate the large spores and hyphae from

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Contribution No. 591 from the Forest Biology Division, Research Branch, Department of Agriculture, Ottawa, Canada.
[2]Forest Biology Laboratory, Quebec, P.Q.

the minute endospores. A maximum concentration of endospores was obtained by breaking hyphae and spores in a blender for 30 to 60 seconds before centrifuging.

Staining of fresh material was carried out according to standard techniques. Several vital and cytoplasm stains, including neutral red, Sudan IV, brilliant vital red, crystal violet, cotton blue in lactophenol, phloxine, and Janus green B, were used.

TABLE I

Source of cultures*

| Culture No. | Date isolated | Locality |
|---|---|---|
| Q1 | Dec. 10, 1958 | Quebec City, P.Q. |
| Q2 | Dec. 2, 1958 | St. Romuald, Levis Co., P.Q. |
| Q3 | Dec. 15, 1958 | Sherbrooke, Sherbrooke Co., P.Q. |
| Q4 | July 1958 | St. Sulpice, L'Assomption Co., P.Q. |
| Q5 | July 1958 | Ste. Théodésie, Verchères Co., P.Q. |
| Q9 | Mar. 12, 1959 | Montréal, P.Q. |
| 3 | June 1958 | Montréal, P.Q. |
| NB100 | June 1958 | Aroostook, Victoria Co., N.B. |
| NB101 | Aug. 9, 1958 | Woodstock, Carleton Co., N.B. |
| T204 | Summer 1958 | Setauket, New York, U.S.A. |
| T236 | Summer 1958 | Rochester, New York, U.S.A. |
| T307 | Summer 1958 | Proctor, Vermont, U.S.A. |
| 57-1435 | — | Seekonk, Massachusetts, U.S.A. |
| 57-7835 | — | Duxbury, Massachusetts, U.S.A. |
| 58-726 | Apr. 15, 1958 | Florida, Massachusetts, U.S.A. |
| 56-14671 | Dec. 18, 1956 | Bedford, Massachusetts, U.S.A. |
| C | — | Connecticut, U.S.A. |
| 57-1 | — | Hamilton, Wentworth Co., Ont. |
| 57-18 | — | Brantford, Brant Co., Ont. |
| 57-50 | — | Esquesing Twp., Halton Co., Ont. |
| 57-68 | — | Niagara Falls, Welland Co., Ont. |
| 57-119 | — | Niagara Falls, Welland Co., Ont. |
| 57-17 | — | Etobicoke Twp., York Co., Ont. |

*Cultures 3, NB100, and NB101 were used in basic studies either as mass or monoconidial isolates; others were used for comparative studies.

Preliminary nuclear studies were made using smears obtained from liquid cultures of different ages, and on 1-day-old cultures growing on slides coated with a thin film of agar. The material was fixed with a 1% solution of chromo-acetic acid, dried at 60° C for 1 hour, and washed in tap water for a minimum of 30 minutes; in some cases 70% alcohol was used. Nuclear stains included Feulgen's reagents, Heidenhain's iron haematoxylin with eosin, and phloxine with methylene blue. The two former stains were used according to standard procedures; the latter, according to a modification of the method described by Gurr (4). Controls for the Feulgen test were established by omitting hydrolysis with HCl.

Ordinary and phase contrast microscopes were employed in these studies. The photomicrographs were taken on 35 mm Panatomic X Kodak film, using a green filter. The photographs were all taken with the 97× objective, but for convenience were printed at different magnifications.

## Morphological Studies

Microendospores[3] could be observed in typical spores and hyphal cells of *C. ulmi* when still less than 0.5 $\mu$ in diameter, that is, the limit of precise measurement. In young cultures, typical conidia and hyphae appear very dense and show uniform refraction prior to the appearance of microendospores. Concomitantly there appear one or more vacuole-like lighter areas which may or may not contain some of the microendospores (Fig. 1). Subsequent development of typical spores and hyphae results in an enlargement of these vacuole-like areas and an increase in the number of microendospores, as many as 20 having been counted in some spores (Fig. 2B). Often two enlarging vacuole-like areas come in contact within spores and then such spores appear to be two-celled (Fig. 3B). In some spores a vacuole-like area develops in which no endogenous particles are visible; these spores appear to have a very thick wall (Fig. 4). Microendospores are fewer and are less apparent in young, rapidly developing spores and hyphae. Thus, most of the various stages of microendospore development have been observed in older cultures.

Microendospores may develop in a number of ways. They have been observed budding or dividing which gave them the appearance of bacteria (Figs. 3A, 5B) and they have been observed giving rise to small short hyphae (microhyphae) when free or when still enclosed in spores and hyphae. Such microhyphae grow through the wall of or remain confined within the enclosing cell, subsequently producing microendospores and terminal spores which eventually develop into typical spores (Figs. 6–12, 25–28). Microendospores by some means yet undetermined may pass directly through the wall of the enclosing spore or hypha (Figs. 2, 9, 13, 27). The penetration of microendospores through the cell wall, or their development in close contact with it, may be responsible for the knobby appearance sometimes observed on spores, hyphae, and conidiophores (Figs. 12, 14).

Microendospores may be liberated by means of the microhyphal and spore growth through the walls as described above, and by a breakdown of the enclosing cells. These cells then appear to have a thin wall which disintegrates and a very granular cytoplasm that leaks out together with the microendospores into the surrounding medium (Fig. 15).

Finally, the enclosed or liberated microendospores may enlarge, though very slowly, into typical spores (Figs. 3, 9, 11, 12, 16, 17–22, 27). When developing within large hyphae the spores retain a more or less spherical shape; within smaller hyphae they become more oval-shaped by compression of the surrounding walls (Fig. 3B). These typical spores subsequently produce microendospores, thus initiating another cycle. An interesting feature seen in some of the developing microendospores is illustrated in Figs. 16 and 22, which show wing-like appendages attached to the spores. These appendages are thought to be formed by remnants of the partially collapsed walls of the enclosing spores (Fig. 5A).

[3]The term microendospore is used herein to designate minute cytoplasmic particles which were found capable of developing into the typical hyphal and spore forms from which they arose.

All 23 isolates examined, either as mass or monoconidial cultures, showed a similar behavior in the development of microendospores.

An attempt was made to characterize the microendospores by their affinity for various stains. Great variation in staining was observed. It was found that the intensity of reaction increased with the age of the microendospores, except when using Sudan IV which showed an opposite trend. Of several cytoplasm and vital stains employed, cotton blue, phloxine, neutral red, and crystal violet gave the best results. With the Feulgen test, the microendospores stained bright red, but when older, they showed an inner colorless area (Figs. 3, 5, 7, 19–21). A similar reaction was obtained in the controls, although the color was less intense. Using phloxine with methylene blue as a counterstain, results were inconsistent; some microendospores stained red or blue, others took both stains. With haematoxylin and eosin they appeared as refractive brown particles outlined by a pale red band (Fig. 23).

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FIG. 1. 650×. Microendospore isolate from culture Q2, on PDA, examined with phase contrast. Formation of microendospores and vacuole-like areas after 2 days of incubation at room temperature.

FIG. 2. 650×. Culture T307, on PDA slants, unstained. (A) Growth of one microendospore through the wall of the mother spore. (B) Five visible microendospores in a typical spore.

FIG. 3. 650×. Culture NB101, 3-week-old liquid culture, Feulgen. (A) Budding microendospores within hypha. (B) Microendospore that developed into a large spore, appearing to be two-celled.

FIG. 4. 900×. From culture T307, on PDA slants, unstained. Spores appearing to have a thick wall, due to the enlargement of a clear vacuole-like area.

FIG. 5. 800×. Culture NB101, 3-week-old liquid culture, Feulgen. (A) Microendospore outgrowing its enclosing spore. (B) Budding microendospores in hypha.

FIG. 6. 900×. Culture NB101, 1-week-old liquid culture, phloxine. Microhypha growing through the wall of a large spore (see also Fig. 25).

FIG. 7. 900×. Culture NB101, 3-week-old liquid culture, Feulgen. Germ tube from microendospore growing through the wall of a large spore (arrow); other microspores free, or within spores.

FIG. 8. 900×. Culture NB101, 1-week-old liquid culture, Janus green B. Microhypha growing through the wall of a typical spore, and giving rise to a terminal spore. Also in the same spore, other microendospores have grown into small filaments; some of these intermix giving the impression of a spore (see also Fig. 26).

FIGS. 9–12. Culture NB101, 1-week-old liquid culture, phloxine (Fig. 12, Janus green B). Fig. 9. 800×. Free microendospores in development; microhyphae growing within or crossing the walls of the enclosing hyphae; microendospore growing directly through the wall of a large spore (at the bottom left of figure). See also Fig. 27. Fig. 10. 600×. Microhyphae growing through the walls of large hyphae (see also Fig. 28). Fig. 11. 800×. Microhyphae within hypha; free microendospores developing. Fig. 12. 900×. Knobby appearance of a spore due to a microendospore developing near its wall; another microendospore in this spore gave rise to a microhypha at the tip of which a large spore has developed.

FIG. 13. 900×. Culture T307, on PDA slants, unstained. Microendospores growing through the wall of the enclosing spore; a larger spore has formed at the tip of this microendospore.

FIG. 14. 900×. Culture NB101, 1-week-old liquid culture, phloxine. Knobby appearance of the conidiophore. One of the sterigma-like projections at the right appears perforated at its tip.

FIG. 15. 650×. Culture NB100, 1-week-old liquid culture, phloxine. Disintegrating spores.

FIG. 16. 900×. Culture 57-1, PDA slant grown at room temperature for 5 days, kept at 4.5° C for 2 months, and then brought back at room temperature for 1 more week. Mature microendospores within hypha staining heavily with cotton blue, others remaining colorless; wing-like appendage on some spores nearby.

PLATE I

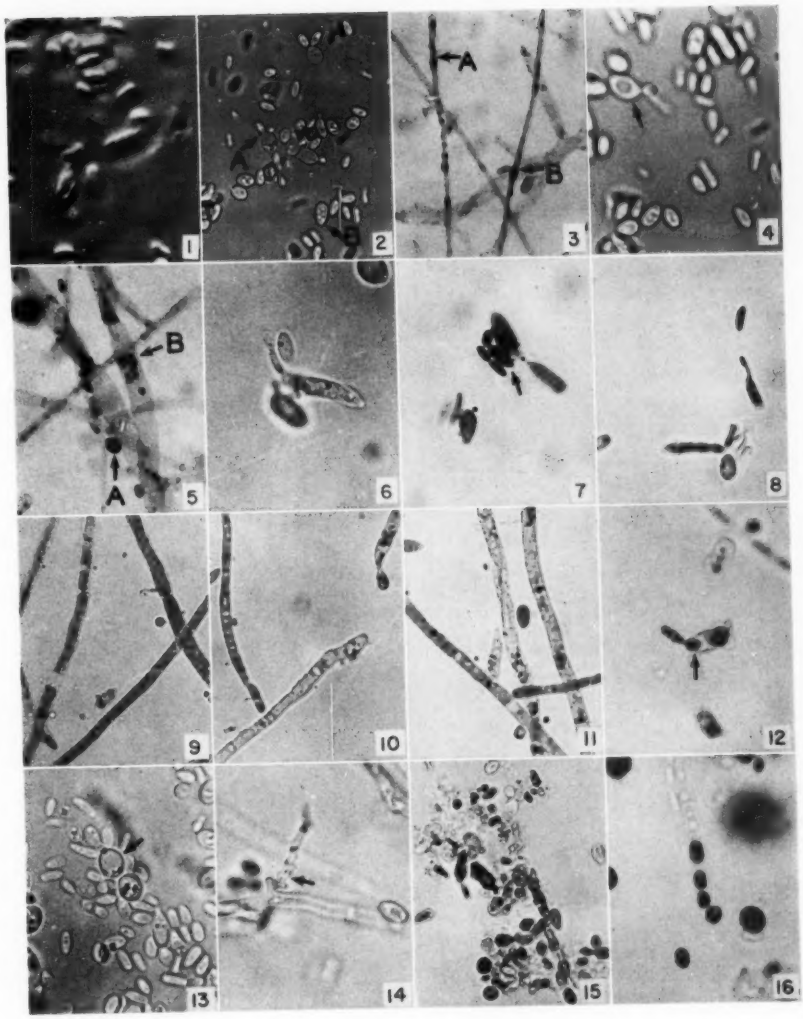


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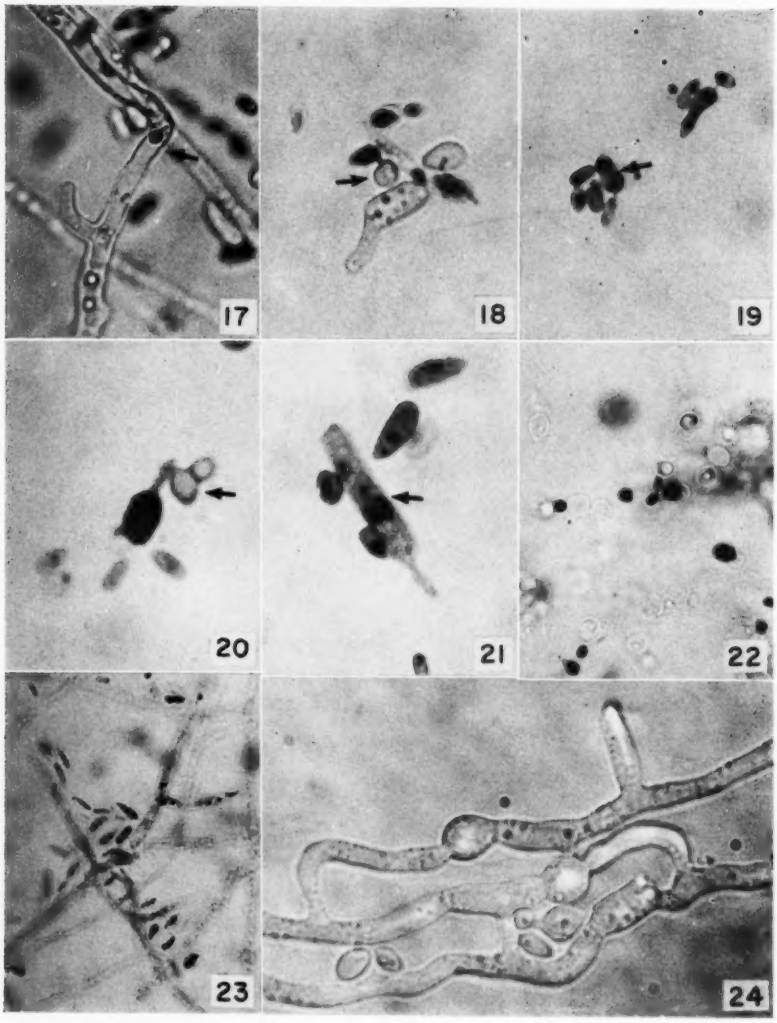
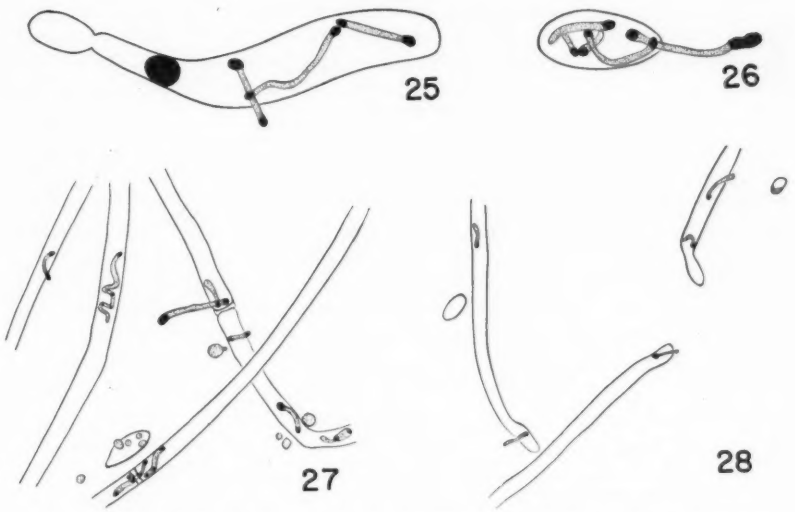
PLATE II






FIGS. 25–28. Schematic drawings illustrating some of the features shown in Figs. 6, 8, 9, and 10 respectively. Figs. 25–26, approximately 2000×; Figs. 27–28, approximately 1200×.

An attempt to demonstrate the existence of microendospores was made by diluting, in a drop of water, a loopful of the supernatant obtained by centrifugation of a spore suspension, and streaking this on PDA in petri dishes. Previously, the inoculum had been checked microscopically for the absence of large spores. With this procedure, and using cultures of Q2, Q5, and NB101, an average (replication of six plates) of 162, 160, and 4.2 colonies per plate were obtained respectively. In a similar experiment, with cultures of Q2, 56-14671, and C, an average of 3, 29, and 55 colonies per plate were counted respectively. An attempt to follow the development of microendospores on the surface of agar was made by periodic observations of the underside of petri dishes using the low power objective of the microscope. Since spores were not seen until 2 days after inoculation of the plates, it was not possible to isolate with certainty single microendospores. These results, however, further indicate that microendospores may develop freely.

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FIGS. 17–18. Culture NB101, 1-week-old liquid culture, phloxine. Fig. 17. 1000×. Developing microendospore in hypha. Fig. 18. 1800×. Similar microendospore developing freely.

FIGS. 19–21. 1800×. Culture NB101, 3-week-old liquid culture, Feulgen. Fig. 19. Microendospore developing into a large spore within a typical spore. Compare with Fig. 12. Fig. 20. Two microendospores in close contact developing freely. Fig. 21. Two microendospores in close contact developing within a spore.

FIG. 22. 900×. Culture 57-1 (see legend for Fig. 16). Wing-like appendages attached to developing microendospores.

FIG. 23. 900×. Culture NB101, 3-week-old liquid culture, haematoxylin and eosin. Refractive brown microendospores outlined by a paler red band.

FIG. 24. 900×. Culture NB101, 3-day-old hanging drop culture. Anastomosis of hyphae.

The time required to complete a cycle in the development of microendospores was not determined, owing to the difficulty of maintaining slide cultures in a condition suitable for observations over long periods.

## Discussion

To the authors' knowledge no report on the production of microendospores in *C. ulmi* as such has been published previously. The particles described in this paper as microendospores may correspond in part to the "oil droplets" mentioned by Schwarz (6) and the "glistening bodies" observed by Clinton and McCormick (3). The presence of microendospores in diseased tissue may have led Brussof (1) to believe that the disease was caused by a bacterium. Wollenweber and Stapp (10) have also observed strongly refractive bodies in diseased tissues and concluded that these were different from bacteria.

The reaction of the microendospores to Sudan IV may indicate the presence of a fatty substance in the walls of young microendospores. The observed change in permeability in aging microendospores as seen with other stains may result from alteration of such a substance. The decreasing intensities in color in developing microendospores obtained with the Feulgen test, before or after hydrolysis, may also indicate progressive changes of certain substances that may polymerize to give greater rigidity to the wall. Substances of an aldehyde nature could be involved.

The budding phenomenon described in *C. ulmi* by Schwarz (6) and others (3, 9, 10) may be partly due to the movement or growth of microendospores through the walls of typical spores as seen in Figs. 2A, 9, 12, 13, 27. Also, the great variability in size and shape of spores observed by these investigators may be explained by the presence of microendospores of different stages of development. The origin of repeated variants obtained from serial monoconidial isolates by some workers (7, 8), which has been attributed to "constant mutations" by Walter (8), may be explained by the existence of microendospores. Walter supposed that spores and hyphal cells were uninucleate, but the presence of several microendospores in such cells suggests, at one stage at least, a multinucleate condition. It is quite possible that these investigators transferred spores containing microendospores of different genotypes, since anastomosis between hyphae has been observed frequently in all the cultures examined (Fig. 24). In addition, the apparent fusion between microendospores as can be seen in Figs. 20 and 21 may suggest a type of sexuality.

Since particles less than 0.5 $\mu$ in diameter, which was the limit of precise observation, were observed to behave like spores, and since the nuclear studies did not provide conclusive results, it has been impossible to identify the nuclei in the spores and hyphae of the organism.

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# VEGETATIVE PROPAGATION IN LINARIA VULGARIS[1]

TRILOCHAN S. BAKSHI[2] AND ROBERT T. COUPLAND

## Abstract

Vegetative propagation in *Linaria vulgaris* is obtained by the development of numerous adventitious buds on its roots. Each bud originates exogenously on the parent root, and it is always associated with a lateral root which arises in the pericycle opposite a protoxylem point. Buds capable of vegetative propagation are not found on either the hypocotyl or the stem. This appears to be correlated with the absence in roots of the typical open type of lenticels and of any significant accumulation of starch. Soil disturbance seems to have a deleterious effect on the production of buds on roots.

## Introduction

It is widely recognized that of all the biological features which influence frequency of a species, capacity for propagation and dispersal are of prime importance. Many weeds, particularly the persistent perennial species, reproduce both sexually and asexually. Therefore, the potential value of either method of reproduction deserves careful study. Reproduction of weeds by means of seeds has been the subject of numerous investigations but their vegetative multiplication has received scant attention. Since anatomical studies of vegetatively multiplying organs are of primary importance to a thorough understanding of the problem of weed propagation (see ref. 1), the writers have undertaken a project to study the comparative anatomy of some of the more important weeds of Saskatchewan. The present work is the second in the series and deals with the subterranean organs of *Linaria vulgaris* (L.; Mill.) Hill.

Vegetative propagation in *L. vulgaris* is obtained by the development of numerous buds on its roots. It is these buds which, along with the "prolific production of strongly-winged and readily dispersed seeds", make the species "a potential menace to large parts of western Canada" (6). The primary stem is entirely vegetative but the main root and most of the laterals produce numerous adventitious buds. According to Salisbury (14), "a typical specimen had by its second year of growth produced no less than 193 adventitious shoots." In Saskatchewan, even a 2-month-old plant may produce buds on roots, and a 2-year-old individual may have as many as 257 adventitious shoots (19). In the material examined by the writers, buds capable of vegetative propagation were found only on roots. The basic morphological problem in the vegetative propagation of the species, therefore, concerns a study of the lateral meristems in its roots and the differentiation and emergence of primordia originating from these meristems.

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[2]Present address: Rice Research Institute, Department of Agriculture, Njala, Sierra Leone, British West Africa.

Few researches on *L. vulgaris*, other than those on its taxonomy and control, have been reported, its importance as a weed notwithstanding. Salisbury (14, 15) worked out its reproductive capacity, and Korsmo (10) has described the detailed anatomy of its vegetative organs. The ecological life history of the species has been recently studied by Zilke (19). Apparently no anatomical work has so far been published on the origin and development of buds on its roots.

## Materials and Methods

The subterranean organs of *L. vulgaris* were collected 6 miles east of Arma, Sask. (NE5-42-2W3), on October 23, 1958, from holes dug about two meters deep. Collections were made from a cultivated field as well as from undisturbed soil of the road-allowance, the two locations being 7 meters apart. Material was fixed in the field in formalin – acetic acid – alcohol, and organs from various depths were stored in separate containers for the purpose of comparisons. The usual methods of dehydration and infiltration were followed. Sections were cut 12 $\mu$ thick. Two combinations of stains, as described by Johansen (8), were used. Of these safranin and fast green gave better results than crystal violet and erythrosin. It was found that the picro-alcohol of Johansen (8) destained the sections in one second to such an extent that almost all the lignified cells appeared light pink. Johansen's technique was, therefore, modified by using one drop of his picro-alcohol in a Coplin jar (115 cc) of 95% ethyl alcohol. Best contrast in staining was obtained when the sections were immersed in safranin for 135 minutes, destained to the required intensity in the modified picro-alcohol (approximately 5 seconds for woody and 2 for non-woody material), and counterstained with fast green for 3 seconds.

## Observations

*Root*

The transection of a young root shows the usual three regions: epidermis, cortex, and stele. Epidermis consists of rectangular or oval cells with yellowish or reddish-brown cuticle on the outer walls. Cortex comprises three to five layers of rectangular cells of which the innermost, the endodermis, usually possesses Casparian strips. In some sections the inner and the radial walls of endodermis appeared to be somewhat thickened. In such cases Casparian strips were not visible. The remaining two to four layers of cortex consist of rectangular parenchymatous cells arranged in almost perfect concentric circles. The cells of the subepidermal layer do not in any way differ from those of the rest of the cortical parenchyma. Stele is made up of the usual tissues of xylem, phloem, cambium, and pericycle. The arrangement of xylem may be either diarch or tetrarch. Diarch xylem, with or without a vessel in the center, is almost the rule in young roots. In older organs, however, tetrarch arrangement is as common as the diarch. There is hardly any starch in the young roots.

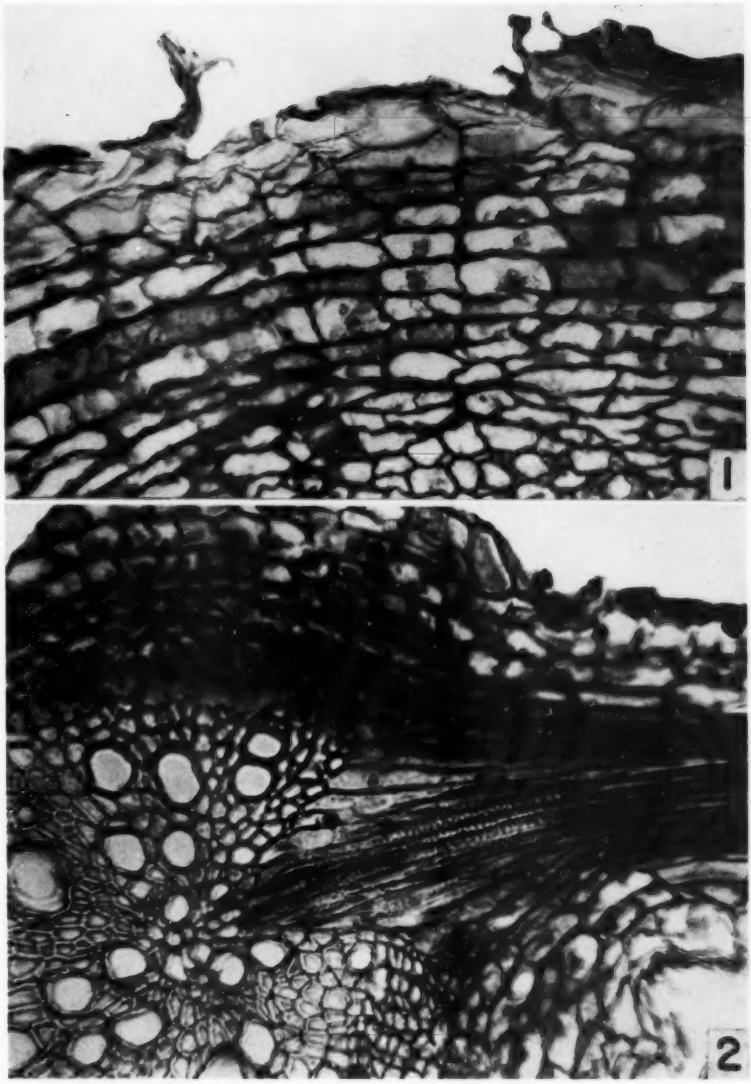
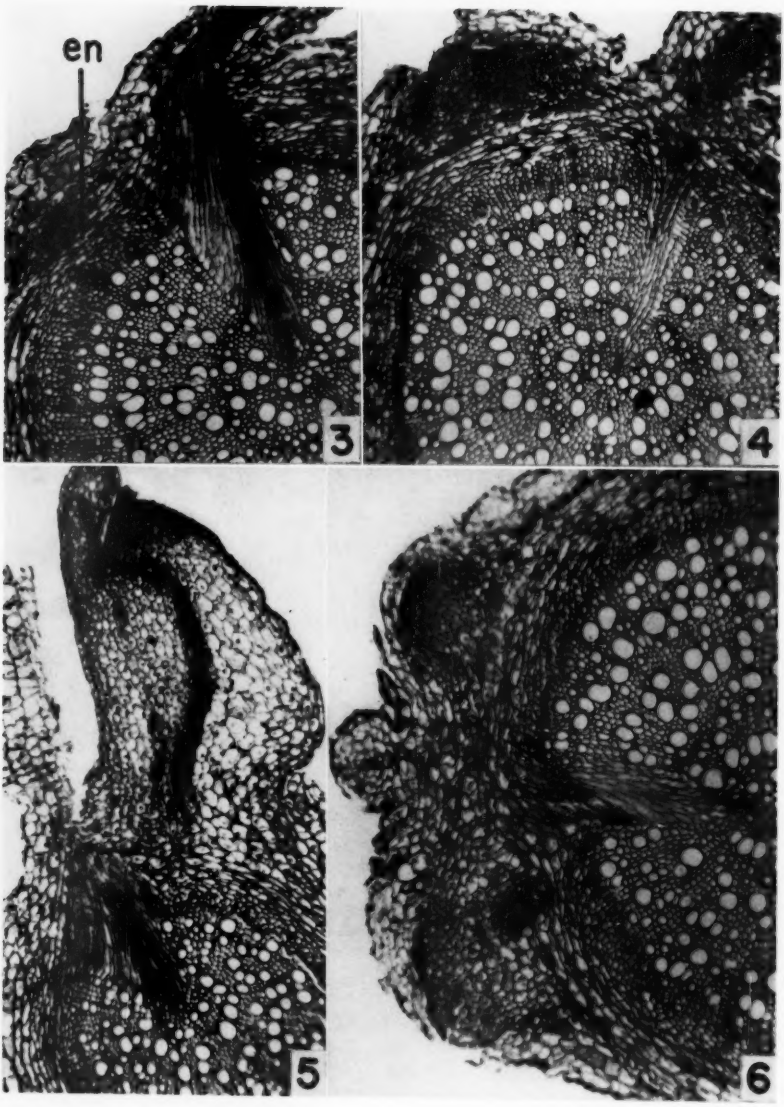



FIG. 1. Part of transection of a 2-year-old root showing cortical origin of phellogen, and epidermal crack. ×260

FIG. 2. Transection of root. The lateral root, cut longitudinally, has originated from the pericycle opposite the protoxylem. ×260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The present study has confirmed Korsmo's (10) account of the secondary growth in the stele of the roots of *L. vulgaris*. An interesting feature of wood is the total absence of vascular rays, or of any other kind of parenchymatous cells. The oldest roots that the writers investigated showed four annual rings, and even in these the endodermis and the cortical parenchyma were found to be intact. As compared with *Euphorbia esula* (1), there is very little, or hardly any, starch accumulation in the roots of *L. vulgaris*.

In the 2- or 3-year-old roots of *L. vulgaris*, either the subepidermal layer or the one immediately below it becomes meristematic, and it gives rise to phellogen which produces one to two layers of cork towards the outside (Fig. 1). Korsmo (10) has not shown the place of origin of phellogen in his illustrations of the roots of the species, nor has he made any reference to it in the text. He does state, however, that "it (the cortex) is covered by a thin layer of cork." Esau (5) has stated that although "a phellogen arises among the outer cells of the proliferated pericycle . . . dicotyledonous roots with a limited amount of secondary growth . . . may develop an exodermis or a superficial periderm." Dicotyledons in which cortical origin of phellogen in roots has already been reported include three species of *Gerardia* (2), *Buttonia natalensis* (3), *Citrus sinensis* (7), *Convolvulus arvensis* (9), *Veronica fruticans* (12), and *Asclepias incarnata* (13).

In sections of older roots, numerous epidermal cracks in various stages of development are visible. They do not seem to be distributed in any definite relation to the inner tissues of the root. They give the suggestion of lenticels but lack the usual several-layered margins as well as the complementary cells of typical lenticels. The single- or double-layered cork is thus directly exposed to the outside through the epidermal cracks.

Soon after the beginning of secondary growth, cells of pericycle opposite one or more protoxylem points become meristematic. Primordia of lateral roots arise from these meristematic points. Lateral root primordia, as evidenced by the crushed or distorted shape of cortical cells of the parent root, push their way through the cortex. Tissue differentiation within the lateral root primordium begins before its emergence from the parent root. Lateral roots are thus endogenous in origin (Fig. 2).

When the parent root is at least one year old, some of its cortical cells adjacent to the lateral root with fully differentiated primary tissues become meristematic (Fig. 3). They divide and redivide to form a well-defined primordium which pushes its way through the cortex of the parent root. Initiation of recognizable tissues within this primordium sets in at about the stage

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FIGS. 3–5. Transections of roots from disturbed soil.

FIG. 3. Origin of shoot primordium (left) in relation to the lateral root. *en* = endodermis. ×70

FIG. 4. A stage more advanced than that in Fig. 3. Note the beginnings of leaf primordia on the shoot primordium. ×70

FIG. 5. Shoot primordium has given rise to adventitious bud. The associated lateral root (left) has been cut tangentially through the cortical parenchyma. ×70

FIG. 6. Transection of root from undisturbed soil. The lateral root (left center) has a shoot primordium on either side. ×70

when its tip is abutting on the subepidermal layer, and by the time it has pushed out through the epidermis several protuberances representing the beginnings of leaf primordia have appeared on its surface (Fig. 4). The shoot primordium, therefore, originates exogenously in the parenchyma of the primary cortex of the parent root and in the tissue juxtaposed with the lateral root (Fig. 5). The writers never found a shoot primordium originating in a parent root tissue lying away from the lateral roots.

It is interesting to note that roots of about the same size but collected from different depths and localities showed similar anatomical features.

Korsmo (10) found that in his material of the subterranean organs of the species there were present "tubers, of the size of pea, solitary or in small clusters." In some tubers there was "a species of snout-beetle, *Gymnetron linariae*." Korsmo could not determine "whether these tubers are caused by the attacks of the insect or if they are primary organs." The "tubers" and the "insect" were absent in the material examined by the writers.

*Stem*

The primary and secondary structure of stem has been adequately described by Korsmo (10). Root-to-stem transition takes place in the region 2–5 cm below the soil surface. The transitional region does not produce any lateral organs. It appears that the stem takes no part in the vegetative propagation of the species. The only buds produced by the underground portion of stem are those lying in the axils of scale-leaves. These buds originate exogenously. Adventitious roots with endogenous origin may be present at the nodes. Like roots, underground portions of stem accumulate very little starch.

## Discussion

Anatomical features which appear to be significant with respect to the control of *L. vulgaris* include the formation of a few-layered cork, the accumulation of little, if any, starch in the cortical parenchyma, and the characteristic development of lateral organs.

Since the roots of *L. vulgaris* lack a well-developed cork, their inner tissues are less protected against the environment than are those of *Euphorbia esula* (1). Lugovoy (11) studied the correlation between type of lenticel development and ability to root as stem-cuttings of a number of tree and shrub species. He concluded that species characterized by a particular open type of lenticel were readily propagated by stem-cuttings. The absence of the open type of lenticels and of vegetative propagation by stems in *L. vulgaris* is, therefore, significant.

Yerkes, Scott, and Swingle (18) have observed that success or failure as understocks in "commercial rose growing" is dependent upon the stage of maturation of the stock at the time of digging, and that many cases of unsatisfactory results reported with apparently well-grown "manetti stocks were really due to digging in an immature condition." They found that abundant

starch signifies satisfactory maturity. The absence of abundant starch accumulation in the subterranean organs of *L. vulgaris* during any period of its vigorous vegetative growth, and of vegetative propagation by its stems is, therefore, of considerable significance.

It has already been shown by the writers that the presence of a continuous ring of pericyclic meristem adds considerably to the potentiality of *Euphorbia esula* in the production of lateral organs (1). That *L. vulgaris* does not possess such a ring of pericyclic meristem, therefore, becomes significant in view of the observation that in the uppermost region of the soil, whereas an average of 42 buds per 15 cm length of a given organ (either root or stem or both) are produced in *E. esula*, only 25 are formed over the same length of an organ (roots only) in *L. vulgaris*.

According to Willis (17) each year's growth of *L. vulgaris* arises "from an adventitious bud upon the summit of the root." It is not clear from this statement whether Willis is referring to "the summit" of every root or of only the main root. The writers have found that the buds originate on roots of all orders and that in every case they arise in the tissue juxtaposed with a lateral root of the next order. Unlike certain other species of *Linaria* (4, 13), *L. vulgaris* does not produce adventitious buds on its hypocotyl or stem although these organs may produce adventitious roots.

Priestley and Swingle (13) have gone to great length to explain the causes of the origin of adventitious buds in connection with the lateral roots. They believe that as the lateral root primordium pushes its way through the parent root cortex, the endodermis breaks, and there occurs a leakage of water and nutrients from the stele into the cortex. This leakage is believed by them to be instrumental in changing the pH of the cortical tissue with the consequent stimulation of meristematic activity in this region. Several exceptions to these broad generalizations are noted by them, and they conclude that "callus and bud formation at the base of branch roots have now been traced to the presence of a break in the endodermis at this point, and the absence of the buds and callus in many cases is apparently due to endodermal continuity." Tetley (16) found a similar situation in the Compositae where the stele releases unusual quantities of fat and fat-soluble substances which collect in the intercellular spaces of the cortex. The presence of this "intercellular secretory system" stimulates meristematic activity in the cortex.

The writers' study of *L. vulgaris* does not seem to confirm the "leakage" hypothesis of Priestley and Swingle (13). The endodermis of the parent root in *L. vulgaris* has been found to be continuous with that of the lateral root. The "leakage" from the stele to the cortex does not, therefore, appear to be of any great consequence, at least in *L. vulgaris*. This is further confirmed by the observations that there are no intercellular secretory pockets in the cortex of this species, and that the usual fat stains, like Sudan III which was also used by Priestley and Swingle (13) and Tetley (16), did not show the presence of fats in any of the cortical intercellular spaces. Sudan III did, however, give a positive reaction in the stelar region.

For a thorough understanding of the persistence of perennial weeds, all aspects of the production of adventitious buds by their subterranean organs must be fully investigated. The writers suggest that in any such study the hormonal aspect should not be overlooked. For instance, in the present case it is possible that a lateral root produces certain hormones not produced by the parent root but necessary for the stimulation of cortical tissues for the production of exogenous adventitious buds. That the parent root tip may be producing such hormones does not appear to be possible, at least in *L. vulgaris*, for two reasons. First, several lateral roots nearest the parent root tip do not have any buds associated with them, and second, the buds are not initiated in the cortex of the parent root until the primary tissues in its associated lateral roots have been fully differentiated.

Although roots from both the cultivated and undisturbed land did not show any significant anatomical variations, interesting information has been obtained with respect to the number of lateral roots and buds per unit length of roots from the respective areas. Fifteen root specimens from each location were examined. It was found that in the cultivated soil there was an average of 53 lateral roots per 15 cm length of the main root, and in the undisturbed soil the average number of lateral roots for the same length of the main root was 67. Again, while each lateral root collected from cultivated soil was always associated with but one adventitious bud, each lateral root in the undisturbed soil was associated with an average of 1.5 buds (Fig. 6). Soil disturbance, therefore, appears to have an adverse effect on the production of adventitious buds.

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# OBSERVATIONS ON CEREAL EMBRYOS IN RELATION TO THE "EMBRYO TEST" FOR LOOSE SMUTS[1]

R. C. RUSSELL


## Abstract

The embryos of wheat, barley, oats, and rye may be distinguished readily by differences in the shape of the scutella and by the relative sizes of the root initials. Photomicrographs of transverse sections of a wheat embryo show that the embryonic leaves within the coleoptile are arranged alternately, the first one facing towards the scutellum. Their edges are curled around the growing point and meet on the opposite side of the cavity in the coleoptile. A structure, referred to as a "pad", covers the side of the embryo facing the endosperm.

Abnormalities found in whole barley embryos include the dormant mycelium of loose smut which appears as brown threads mainly in the surface tissues of the scutellum. Frost, occurring during the later development of the kernel, causes a stunting of the embryo, particularly of the coleoptile, and the production of small particles of yellowish-brown pigment in the tissues of the embryo. Other abnormalities are figured and discussed.


## Introduction

Several good descriptions of the anatomy of cereal embryos have been published. Percival (4) and McCall (3) described that of wheat and Avery (1) dealt with the embryos of maize, oats, and wheat. Both McCall and Avery gave a brief review of previous papers by various authors on the same general subject. Most of these previous descriptions were based on studies of sections of whole grains.

A method of separating wheat and barley embryos from the other parts of these grains was described by Simmonds (8) in 1946. He used it to detect the mycelia of *Ustilago tritici* (Pers.) Rostr. and *U. nuda* (Jens.) Rostr. in the embryos of wheat and barley, respectively. The feasibility of making practical use of this embryo test in forecasting the severity of loose smut infection in barley was investigated by Russell (6) and later by Russell and Popp (7). As a result of their findings, those in charge of barley seed certification in Canada became interested in the test and it is now used throughout this country as a basis for accepting or rejecting barley seed for registration, as far as its loose smut content is concerned. The same method has been used to a limited extent to extract the embryos of oats and rye from the kernels of those cereals.

This paper records and illustrates observations made during extensive studies on cereal embryos. First, there is a discussion of the anatomy of the wheat embryo based on a study of sections of wheat embryos. This is followed by a comparison of the gross appearance of the whole embryos of our common cereals. Finally, a number of abnormalities found in barley embryos are described. Photomicrographs are employed as illustrations.

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Contribution No. 35, Canada Agriculture Research Station, Saskatoon, Saskatchewan.

## Materials and Methods

Sections were made of paraffin-embedded embryos from kernels of Marquis wheat that had been softened in water. They were stained with safranin and light green.

The whole embryos were separated from the other parts of the grain by a modification of Simmonds' method (8). Suitable amounts of dry seed were soaked in a 5% solution of sodium hydroxide for 15 hours at room temperature. As the endosperm absorbed the liquid it swelled and burst the seed coats and hulls. The material was then washed with warm water through two screens and caught in a third screen of fine mesh. The material was washed from the third screen, inverted, into a white tray. The embryos were picked out of the remaining debris and deposited in a small glass beaker, by means of a short piece of glass tubing fitted at the top with a rubber bulb. While any cereal embryo may be separated from the other parts of the kernel by this method, rye embryos are separated most easily and oat embryos offer the most trouble.

The embryos were dehydrated in 95% alcohol after the water was drawn off. They were then cleared in a mixture of equal parts glycerin, phenol, lactic acid, and water. This clearing agent was also used for preservation, but in some cases crystals developed in or on the embryos after prolonged storage.

## Anatomy of the Wheat Embryo

Although the anatomy of the wheat embryo has been described by Percival (4), Avery (1), McCall (3), and others, it is considered advisable to include a description here in conjunction with a comparison of the whole embryos of the four common cereals. In addition, a "pad" which covers the face of the wheat and barley embryos apparently has not been described previously.

The embryo consists of the following parts: hypocotyl, growing point, embryonic leaves, coleoptile, root initials, coleorhiza, scutellum, and epiblast (Fig. 1). The hypocotyl forms the axis of the embryonic plant. There are five or six root initials attached to the hypocotyl and these are enclosed in the coleorhiza. At the upper end of the hypocotyl is the growing point, surrounded by two or three embryonic leaves and the coleoptile. The scutellum is a shield-like structure, attached to the side of the hypocotyl, and it lies between the embryo proper and the endosperm. Rudimentary fibrovascular bundles run through the upper half of the scutellum. On the opposite side of the hypocotyl is a scale-like appendage called the epiblast.

When a whole embryo (Fig. 2A) is examined, the growing point may be seen by carefully focusing the microscope but it is difficult to discern how the leaves are arranged. A longitudinal section through the center of an embryo (Fig. 2B) does little to clarify the matter. However, transverse sections cut at the proper levels in the embryo (Fig. 2, D and E) show clearly that initials of the foliage leaves develop alternately on opposite sides of the axis and that the first leaf faces towards the scutellum. The two edges of the leaf blade curl around the growing point and meet on the opposite side. Both longitudinal and transverse sections through the scutellum (Fig. 2, B–F)

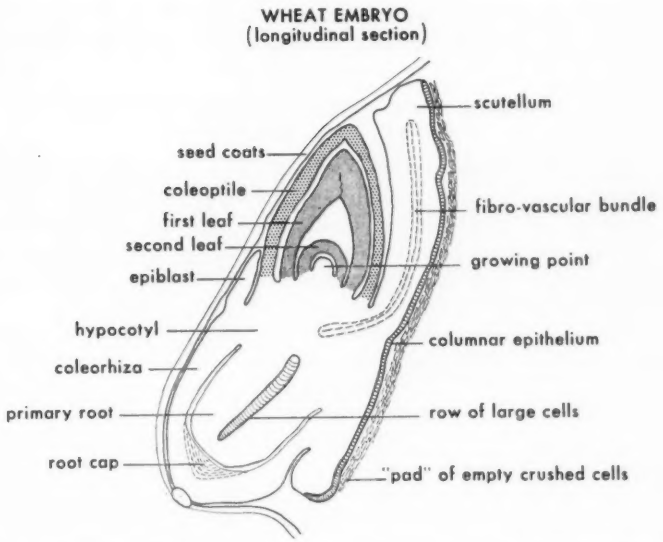


FIG. 1. Longitudinal section of a dormant wheat embryo, with the different anatomical features labelled.

show a row of rectangular epithelial cells which form a layer over the face of the scutellum. They also show a layer of crushed empty cells between the epithelial layer and the endosperm. This is shown most clearly in Fig. 2E. These cells constitute a "pad" (Fig. 1, "pad"; and Fig. 3, E and F). Percival (4) refers to the cells of which the pad is formed as "crushed empty cells of the endosperm", but they seem to have a separate entity. When wheat or barley embryos are separated from the endosperm and seed coats, by Simmonds' method, the pad frequently remains attached to the face of the embryo. When embryos are examined for smut mycelium it is necessary to remove this pad in order to see the tissues of the scutellum clearly. If the embryo is infected with loose smut the mycelium usually passes through the pad on its way into the scutellum and binds the pad to the face of the scutellum. Smut mycelium may be seen very clearly in this thin transparent pad.

Most wheat embryos have five or six root initials. A few additional roots may appear at seed level in the seedling stage, but these are not true seminal roots, as their initials are not visible in sections of the resting embryo and they emerge just above the attachment of the coleoptile (9). The seminal root initials are furnished with root caps and are surrounded by the coleorhiza. The primary root is larger than the others and it is attached to the lower end of the hypocotyl. A continuous row of relatively large cells runs down the center of the primary root (Fig. 1 and Fig. 2B). As these cells mature they form the central tracheal tube of the stele. Two pairs of lateral roots arise in the tissues of the hypocotyl at about the level of attachment of the epiblast.

Diagonal sections of the first pair may be seen in Fig. 2F. In some cases a sixth root emerges just above the epiblast, at right angles to the two pairs of roots (Fig. 2A).

## Comparative Morphology of Embryos of Wheat, Barley, Oats, and Rye

The embryos of the four common cereals differ in general outline and in certain other respects (Fig. 3) but they also show marked similarities. The embryos of durum wheat are usually longer, in proportion to their width, than are those of bread wheats. In barley, and perhaps in other cereals, certain varieties have distinctive outlines and a certain amount of variation is seen in the outline of different embryos of the same variety.

The scutella of embryos of Stewart durum wheat are usually rectangular in outline (Fig. 2A) while those of Selkirk hard red spring wheat are nearly square (Fig. 3A). The first pair of seminal rootlets stand out at a wider angle in the latter. In both kinds of wheat the tip of the scutellum does not extend far beyond the tip of the coleoptile. The numbers of seminal roots may vary from three to six, depending partly on the variety. The second pair after the primary root, when they are present, are usually much smaller than the first pair.

The embryos of barley (Fig. 3B) are usually somewhat larger than those of wheat. The outline varies considerably with different varieties but many of them are ovate. The tip of the scutellum tends to project farther beyond the tip of the coleoptile than in wheat. Generally, five root initials are evident, and the lateral ones slant down at a more acute angle than do those of wheat. Also they are more nearly equal to the primary root in length, and the second pair are more nearly equal in size to the first pair than are those of wheat.

The embryos of oats are distinctly lanceolate in outline (Fig. 3C). The length of the scutellum is far greater than that of the coleoptile. Only three root initials are clearly visible and the lateral ones are much shorter than the primary root. Sometimes a fourth seminal root develops just above the lateral ones.

Rye embryos are rectangular in outline and the coleoptile is relatively long (Fig. 3D). The number of root initials varies somewhat but commonly six are present. The lower end of the coleorhiza is more pointed than in wheat and barley and in this respect resembles the coleorhiza of the oat embryo. The position and size of the root initials appear to be somewhat variable. In some embryos the second and third roots are approximately equal in size and they emerge on either side of the base of the primary root as in the other cereals. In others the second root initial lies directly over the primary root and is noticeably larger than the third.

## Abnormalities in Barley Embryos

The scutellum of a normal barley embryo is fairly clear and transparent after the embryo has been treated with a clearing agent. Any departure from this appearance suggests an abnormal condition. Alterations in appearance

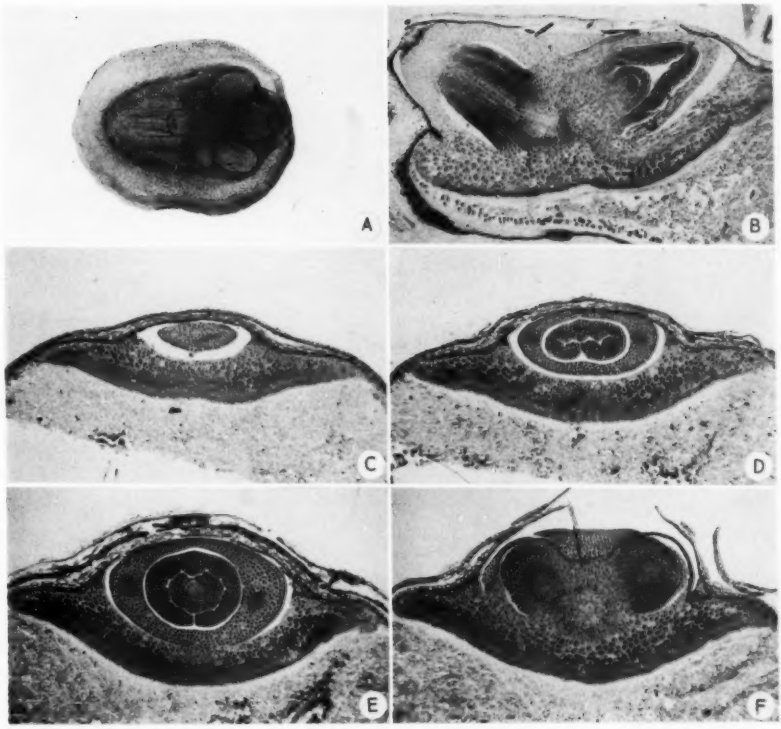


FIG. 2. A—Ventral aspect of a dormant embryo of a durum wheat kernel, showing the growing point and six root initials. B–F—Sections of Marquis wheat embryo: B—longitudinal section (Fig. 1); C—transverse section at the tip of the coleoptile; D—transverse section at the middle of the first leaf; E—transverse section at the level of the growing point, showing two embryonic leaves curled around the growing point; F—transverse section at the level of the hypocotyl, showing the outline of the primary root and the first pair of lateral roots.

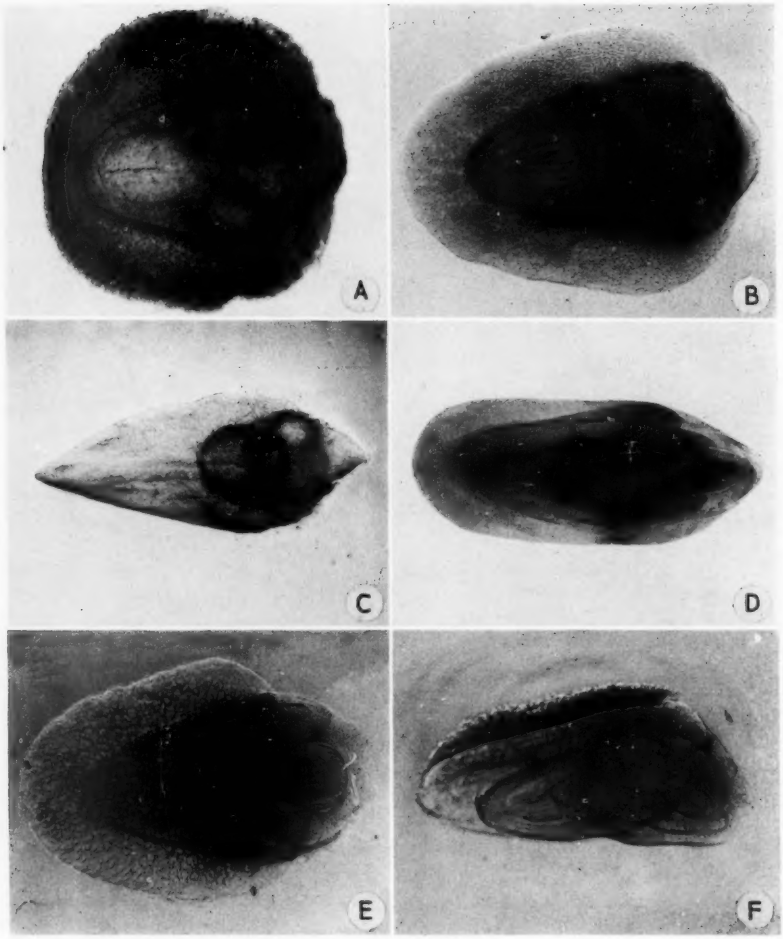


FIG. 3. Cereal embryos, illustrating differences in gross appearance: A—common bread wheat; B—barley; C—oats; D—rye; E—barley embryo with "pad" covering the face of the scutellum; F—side view of the same.

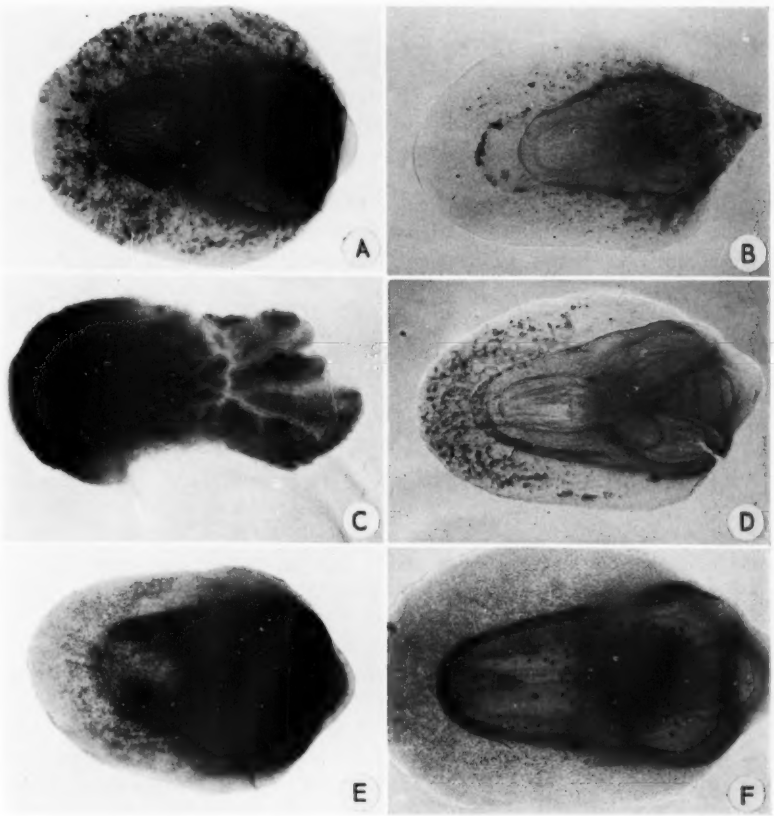


FIG. 4. Abnormalities which may appear in barley embryos: A—dormant mycelium of *Ustilago nuda*; B—symptoms of injury from frost during the ripening period; C—lobulate scutellum; D—"yellow speck"; E—"black speck"; F—rosette-shaped crystals in the tissues of the embryo.

may be seen in the body of the embryo, but they are not so clearly evident because the tissues at the center are thicker. While examining thousands of individual embryos for the presence of the mycelium of *U. nuda*, a number of abnormalities have been noted. The causes of some of these have been determined. Most of them have been studied in detail and photographed (Fig. 4).

The first important abnormality studied was the mycelium of *U. nuda* lying dormant in the tissues of the embryo (Fig. 4A). Batts (2) and Popp (5) have shown that the mycelium of the fungus penetrates the scutellum and passes from it into the hypocotyl and the basal parts of the plumule. However, the development of the mycelium is most abundant in the scutellum and, ordinarily, this is where it is seen when a whole embryo is examined. The mycelium looks like brownish threads or pegs penetrating among the cells of the scutellum from the pad which originally covered its face. The areas affected usually show a brownish-yellow discoloration which aids in detecting small foci of infection. The mycelium is seen more easily in barley embryos than in those of wheat.

When late summer frosts occur before the barley kernels are mature, characteristic symptoms develop in the embryos. These symptoms vary in intensity depending on the stage of maturity and the severity of the frost. In moderate cases (Fig. 4B) the growth of the coleoptile is stunted and it fails to fill the cavity which it occupies. The proximal end of the embryo is more pointed than usual and a yellowish-brown pigment develops throughout the tissues of the embryo, particularly around the margin of the space occupied by the coleoptile. In certain years, when summer frosts are prevalent, these symptoms are found in a large proportion of the seed samples. They indicate that the seed will germinate poorly and that the development of seedlings grown from them may be retarded and the first leaves may be somewhat distorted.

A third abnormality, found in some samples of barley seed, is the presence of lobulate outgrowths of unknown origin at the margin of the scutellum (Fig. 4C). These usually appear at the distal end of the embryo and vary greatly in size and shape.

Another abnormality of unknown origin is called "yellow speck" (Fig. 4D). It appears as specks of bright yellowish pigment scattered through the tissues of the scutellum. This pigment is somewhat lighter and brighter than the discoloration that appears around the foci of smut infection, but its superficial resemblance to loose smut mycelium may cause some uncertainty and confusion.

Abnormalities arising in nature may be confused with other conditions that may develop when embryos are improperly processed for examination. If the tissues are incompletely dehydrated by taking the embryos out of the alcohol too soon, a condition which may be called "black speck" develops. This has the appearance of minute black particles which may be scattered throughout a portion of the scutellum (Fig. 4E) or concentrated in certain areas at the base of the coleoptile or in the coleorhiza over the root initials.

If the same portion of clearing agent is used several times on different lots of embryos, small rosette-shaped crystals are likely to develop in the tissues of the embryo (Fig. 4F). Ordinarily these are concentrated around the embryo proper, but sometimes they permeate the scutellum and interfere with a search for the mycelium of loose smut. They may be eliminated by adding a small amount of distilled water to the material and seldom appear if fresh clearing agent is used for each lot of embryos.

## Discussion

The embryos of wheat, barley, oats, and rye are similar in most respects. Differences in appearance arise mainly from the shape of the scutellum. The general outline of the various embryos, together with characteristic angles at which the lateral root initials grow out from the hypocotyl, make it possible to recognize the embryos of each particular cereal at a glance. This fact helps to avoid confusion, when making routine embryo tests, if more than one cereal is contained in the sample.

The "pad", which covers the face of the scutellum of a wheat or barley embryo (Fig. 3, E and F), is a peculiar structure. It appears constantly in embryo material separated from wheat or barley by the Simmonds' method. It may be that these pads are composed of empty endosperm parenchyma cells whose contents have been absorbed by the maturing embryo. However, in the process of extracting and clearing the embryos, by the Simmonds' method, the pads cling to the embryos and do not remain with the endosperm. In the clearing agent the cells become turgid. Whatever their origin may be, the pads constitute definite structures appearing as thin shields of regular size and shape corresponding to the surface of the scutellum. It is probable that former investigators, using other methods of dissecting out the embryo, have never regarded these cells as forming a definite entity.

The abnormalities described in the body of this paper are the ones most commonly seen during examination of barley embryos in Western Canada. With the increased use of the "embryo test", for the detection of loose smut in seed barley, it is hoped that the description and illustrations given in this paper will prove helpful to those engaged in making such tests.

## Acknowledgment

The author is greatly indebted to the staff of the bio-Graphic Unit, Canada Department of Agriculture, Ottawa, for preparing the illustrations for this paper.

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# A NEW GENUS IN PLANT RUSTS[1]

B. K. Bakshi and Sujan Singh

## Abstract

The life history of *Chrysomyxa piceae* Barclay, the needle rust of spruce, *Picea smithiana* (Wall.) Boiss, has been worked out. The teliospores are one-celled, like *Coleosporium*, but germinate by external promycelia, like *Chrysomyxa*. The rust does not belong to either *Coleosporium* or *Chrysomyxa* or any other known genus and is named *Ceropsora* and described. It is allied to *Mikronegeria*. *Chrysomyxa piceae* Barclay becomes the type species of the new genus and is renamed as *Ceropsora piceae* (Barclay) Bakshi and Singh n. comb.

There are four species of rusts on needles of Himalayan spruce, *Picea smithiana* (Wall.) Boiss. Two of them are *Peridermiums*, namely, *P. piceae* (Barclay) Sacc. and *P. thomsonii* Berk., the third is *Chrysomyxa deformans* (Diet.) Jaczew., and Barclay (1890) described the fourth as *Chrysomyxa piceae* (Fig. 1), which is the subject of the present study. Barclay admitted that his description of *C. piceae* was imperfect. He noticed that teliospores were one-celled (Fig. 2), and not catenulate, so that the rust is more nearly related to *Coleosporium* than to *Chrysomyxa*. Barclay could not germinate the teliospores, so that he included the species under *Chrysomyxa* on general rather than on particular analogy.

Germination of teliospores was obtained in the laboratory from infected spruce needles collected from Chakrata (alt. 6000–7000 ft) in the first week of June, 1959. The germination took place when the needles were kept in a moist chamber and no special technique was necessary. No germination was obtained from specimens collected from Chakrata a week earlier, or from Himachal Pradesh, Western Himalayas, from needles collected in end of May, 1958. Maturity of telia and a brief resting period appear to be necessary for germination.

Germination occurred by apical prolongation of teliospores into the promycelium (Figs. 3 and 4*a*). This suggested that here we are dealing with a rust which possesses one-celled teliospores like *Coleosporium*, but which, unlike *Coleosporium*, germinates by an external promycelium, which is a feature characteristic of *Chrysomyxa*.

Professor Cummins, to whom the specimens of the rust were sent for opinion, agreed that the rust does not belong to either *Coleosporium* or *Chrysomyxa*. He drew our attention to certain similarities between this rust and *Mikronegeria*, a monotypic genus with the type species, *M. fagi* Diet. & Neger, occurring on *Fagus* in South America. Both have large basidia which develop as apical extensions of the teliospores. The basidia in *Mikronegeria* have been described as internal, but this is not correct. The illustration of the telium of

[1]Manuscript received November 24, 1959.
Contribution from the Forest Pathology Branch, Forest Research Institute, Dehra Dun, India.

*Mikronegeria* is reproduced (Fig. 5) by kind permission of Professor Cummins (1959).

However, the present rust is not a *Mikronegeria* and the following essential differences exist between them.

| *Picea* rust | *Mikronegeria* |
|---|---|
| 1. Telia subepidermal, strongly erumpent | 1. Telia subepidermal, rupturing the epidermis but not erumpent |
| 2. Sporogenous tissue well developed | 2. Sporogenous tissue scanty |
| 3. Teliospores laterally coalescent in young sori though separating at maturity | 3. Teliospores laterally free at all stages |
| 4. Peripheral cells present | 4. Peripheral cells absent |
| 5. Sporidia basipetal | 5. Sporidia acropetal |
| 6. Telia on conifers | 6. Telia on hardwoods |
| 7. Uredia unknown, microcyclic | 7. Uredia present, macrocyclic |
| 8. Teliospores cylindric | 8. Teliospores clavate-elliptic |

Professor Cummins agrees with us that the *Picea* rust should not be transferred to *Mikronegeria* but should be established as a new genus, which we name *Ceropsora* and herewith describe.

## *Ceropsora* nov. gen.

*Latin diagnosis.*—Telia subepidermalia, erumpentia, cerea, alte aurantiaco-rubra, convexa vel plana; teliosporae insidentes filamentis longis, multiseptatis aurantiaco-rubris efformantibus massam sporogenam; teliosporae longae, cylindricae, non catenatae, unicellulate, lateraliter adhaerentes inter se in soris novellis, separatae ad maturitatem; nonnullae cellulae peripherales adsunt; promycelium externum, evolutum ex prolatione apicali teliosporae, crassum, alte aurantiaco-rubrum, quater septatum; sterigmata crassa, sporidia globosa, pallide lutea, evoluta basipetaliter. Aecia, uredia atque pycnia ignota. Accedit ad *Mikronegeriam* Dietel, a qua tamen differt teliis erumpentibus, textibus sporogenis bene evolutis, cellulis peripheralibus praesentibus, teliosporis lateraliter adhaerentibus, sporidiis basipetalibus.

Dried and bottled specimens of the type genus deposited in Herbarium and Museum of Forest Pathology Branch, Forest Research Institute & Colleges, Dehra Dun.

(Herbarium 7177, 7178 and Museum 926 and 927.)

The type species of *Ceropsora* is *Chrysomyxa piceae* Barclay, which is renamed *Ceropsora piceae* (Barclay) Bakshi & Singh n. comb. A description of the species is given below.

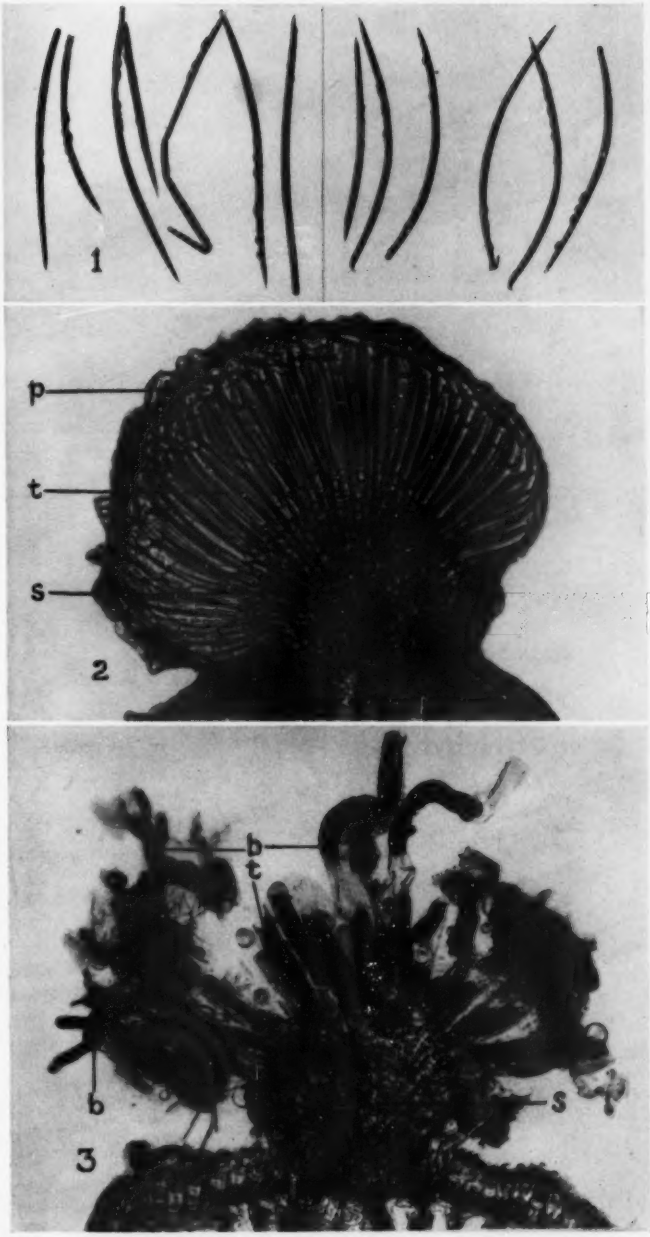
## *Ceropsora piceae* (Barclay) Bakshi & Singh n. comb.

*Technical Description*

Telial beds mainly on upper side of the needles (Fig. 1), telia subepidermal, erumpent, waxy, deep orange-red, convex to flat, formed in a single row,

FIG. 1. Infected needles of *Picea smithiana*, showing telia of *Ceropsora piceae*. ×1.3
FIG. 2. A young telium of *C. piceae* showing sporogenous tissue (*s*), teliospores (*t*), and peripheral cells (*p*). ×125
FIG. 3. A germinated telium of *C. piceae*, showing sporogenous tissue (*s*), teliospores (*t*), and promycelia (*b*) with sterigmata and basidiospore. ×160

PLATE I

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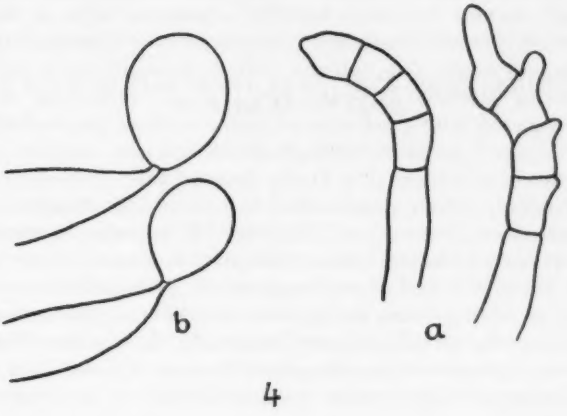


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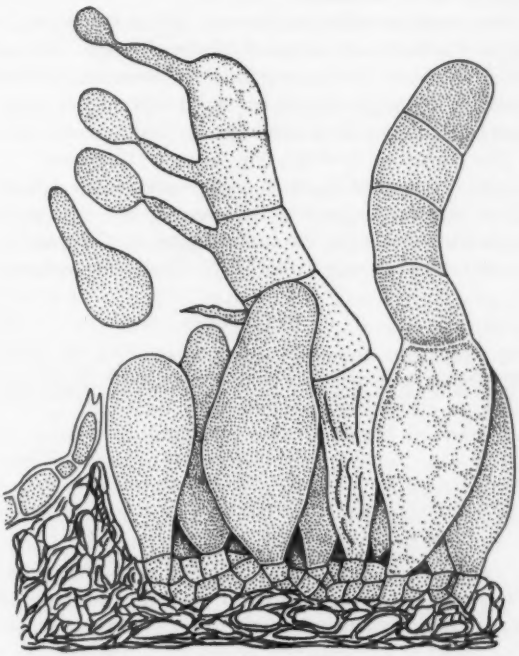

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FIG. 4. *a*, Promycelium and *b*, basidiospores of *C. piceae*. *a*, ×310; *b*, ×1300
FIG. 5. A germinated telium of *Mikronegeria fagi*. ×700

scattered telia found sometimes outside the row, 2–13 on each needle, 2.5–3.0 mm in length and 0.2–0.6 mm in breadth; teliospores borne on long, serially arranged, much septated orange-red filaments forming a compact sporogenous mass; teliospores sessile, long, cylindric, serially arranged, not in chains, single-celled, adhering laterally in young sori (Fig. 2), separating at maturity, contents orange-red, with a well-defined central nucleus, thin-walled, 98–154 × 14–24 $\mu$; a few small peripheral cells present, which may represent a peridium.

Germination of teliospores (Fig. 3) was observed 4 to 5 hours after incubation in moist chamber. During germination, the tip of the teliospore extends to form a four-celled promycelium (Fig. 4*a*). Promycelia external, straight when young, curved like an arch at maturity, four-celled, deep orange-red, stout, 78.4–98.0 × 19.6–22.4 $\mu$; sterigmata stout, given off from near the tip of each cell of promycelium, orange-red, narrowing at the tip into a short hyaline tube bearing sporidia, formed basipetally, that is, the oldest near the tip of the promycelium and the youngest at the base (Fig. 4*a*), 23.6–42.0 × 8.4–10.2 $\mu$; basidiospores light orange yellow, globose to pyriform (Fig. 4*b*), 13–17 × 10–13 $\mu$.

Uredia, pycnia, aecia unknown.

The rust has been recorded on spruce (*Picea smithiana*) from Chakrata division, Uttar Pradesh and Lower Bashahr division, Himachal Pradesh in the Western Himalayas. Young saplings are commonly attacked. Pustules appear on needles of the previous year only, and not on those of the current year. A single row comprised of from two to many telia is usually formed on one needle. The tissues around the telia become discolored.

Grateful acknowledgment is made to Professor G. B. Cummins, for his invaluable advice and for suggesting the name of the new genus, and to Rev. Dr. H. Santapau for rendering the Latin diagnosis of *Ceropsora*. A part of the collection of the rust was made by Sri H. M. Mathur, to whom thanks are due.

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## NOTES

### A NOTE ON ELECTRICAL RESISTANCE MEASUREMENTS IN ACER SACCHARUM

D. S. FENSOM[1]

In the course of studying the biopotentials in plants (1, 2, 3) numerous measurements of potential difference and of electrical resistance have been made on the trunks and branches of maple trees, *Acer saccharum* Marsh., in Ontario at Glendon Hall, Toronto, and at Ridley College, St. Catharines. The patterns of biopotential will be described and discussed in a later paper when other aspects of the study are complete. In the meantime, it would seem that the resistance measurements of the inside of the tree may be useful in other studies of tree physiology, and hence some of these measurements are shown in Fig. 1 below.

To make these measurements, insulated silver nails 1/8 in. diameter were set in the branch under study 3 ft apart (three nails were connected in parallel at each position). The tips of the nails were freed of insulation for 3 mm and were chlorided before insertion in the tree to the depth of the cambium layer. The ambient temperature of the air was also recorded with an ordinary thermometer. In the figure the log resistance is plotted against the air temperature.

As various measurements were taken, it became apparent that there was a correlation between tree resistance and air temperature. A good correlation might persist for several days or even months in which period deviations from close agreement seem to occur only when the air temperature ceased to represent the temperature of the inside of the tree. Over a long period in the springtime, several temperature–resistance correlations appeared to succeed each other in steps. The first step was clearly associated with very cold weather and with high resistance. Since a sharp break in the resistance curve occurred slightly below 0° C, this high resistance is thought to be associated with frozen tissue and could, no doubt, be used to detect the onset of freezing in plant tissues and the nature of partly frozen tissue. The later steps were apparently initiated by periods of warm weather, at least in 1959. Sap flow seemed to be associated with the second step, the opening of buds with the third, and so on. However, throughout the main part of the summer the internal resistance remained both low and less influenced by temperature than in winter or spring. Each of these curves, in the figure where the log resistance – temperature curve approximates a straight line, is the same sort of curve which would result from viscosity–temperature relations in a solution of polyelectrolyte. Hence, studies of resistance and temperatures in plants

[1]Ridley College, St. Catharines, Ontario (Research Associate of the Department of Botany, University of Toronto).

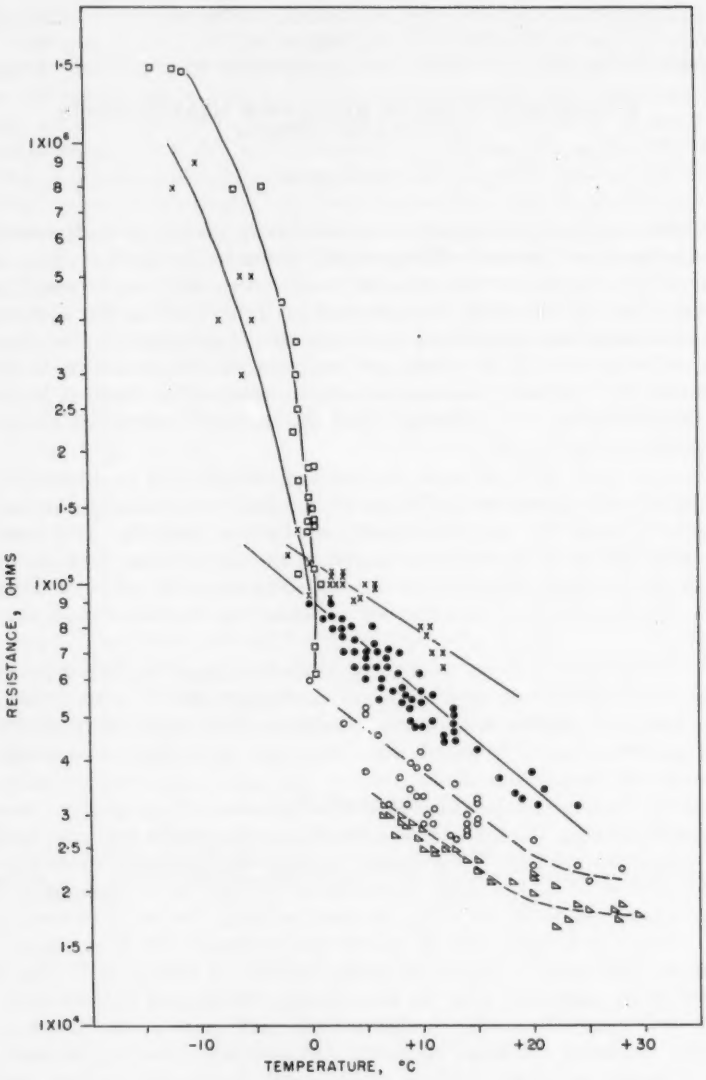


FIG. 1. Log resistance – temperature curves for tree MR51 (St. Catharines) in 1959.

| SYMBOL | TIME OF RECORDING | ASSOCIATED PHENOMENA |
|---|---|---|
| □ | Jan. 1 to March 14 | Frozen tissue |
| × | March 15 to March 28 | Sap flow |
| ● | March 28 to April 18 | Buds opening |
| ○ | April 18 to May 2 | Flowers open |
| ▷ | May 2 to Nov. 1 | Leaves develop and function |

should be useful to detect times of change in tissue activity and to throw light on the movement of charged molecular species through the tissue, or inversely, the movement of liquids through charged pores.

## Acknowledgment

This work was made possible by grants from the Ontario Research Foundation. The assistance of the Dominion Government Laboratory of Plant Pathology in St. Catharines, equipment from the National Research Council, and the comments of Professor D. F. Forward, Department of Botany, University of Toronto, are acknowledged with thanks.

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## NOTE ON A CASE OF LOCALIZED ASYNAPSIS IN TRITICUM[1]

E. B. WAGENAAR[2]

During the study of chromosome pairing in meiotic cells of a progeny of a *T. timopheevi* × *T. durum* hybrid, two florets of one and the same spike showed marked differences in bivalent formation. The plant concerned had 28 chromosomes and one fragment. In one floret, taken from the upper part of the spike, data recorded for 35 cells (Table I) gave an average of 2.5 bivalents and 23.9 univalents (including the fragment). The chromosomes were small and condensed (Figs. 1, 2, and 3). Since this floret was analyzed first, it was thought that all pollen mother cells of this plant would show asynapsis. However, in a second floret, taken from the lower part of the spike, where an

[1]Contribution No. 14 from the Canada Department of Agriculture Research Station, Winnipeg, Manitoba. This study was supported by a National Research Council of Canada Postdoctorate Fellowship.
[2]Postdoctorate Fellow, Cereal Breeding Laboratory, Winnipeg, Manitoba.

TABLE I

Frequencies of metaphase I cells of asynaptic and normal anthers observed in a plant of the offspring of a *T. timopheevi* × *T. durum* hybrid

| Asynaptic anther | | | Normal anther | | |
|---|---|---|---|---|---|
| Chromosome associations | | | Chromosome associations | | |
| I* | II | Frequency | I* | II | Frequency |
| 29 | | 3 | 1 | 14 | 5 |
| 27 | 1 | 4 | 3 | 13 | 12 |
| 25 | 2 | 7 | 5 | 12 | 6 |
| 23 | 3 | 13 | 7 | 11 | 2 |
| 21 | 4 | 8 | | | |
| | Total | 35 cells | | Total | 25 cells |

*Includes one fragment.

average of 12.8 bivalents and 3.4 univalents was recorded, the formation of bivalents was well within the range expected of this progeny (Table I). The chromosomes of these metaphase I cells were of normal size (Fig. 4).

Application of Gaul's formula (1, 3) to the data recorded for the asynaptic cells gave a value of 25.6 chromosomes potentially able to pair. This is a close estimate of the number of pairing chromosomes of the "normal", non-asynaptic cells of this plant. It is thus apparent that the chiasma distribution over the paired chromosomes at prophase I was random, but that the chiasma frequency was considerably reduced in the asynaptic cells (3). A mutation in the somatic tissue producing the pollen mother cells of this anther may have caused this reduction in chiasma frequency.

McCollum (2) recently reported the occurrence of asynapsis in a few panicles of an induced tetraploid hybrid of *Dactylis*. In the present case the asynapsis was probably limited to a single floret or to the upper part of the spike. Consequently, an observed asynaptic effect in one part of a plant does not imply a general irregularity; the effect may be localized.

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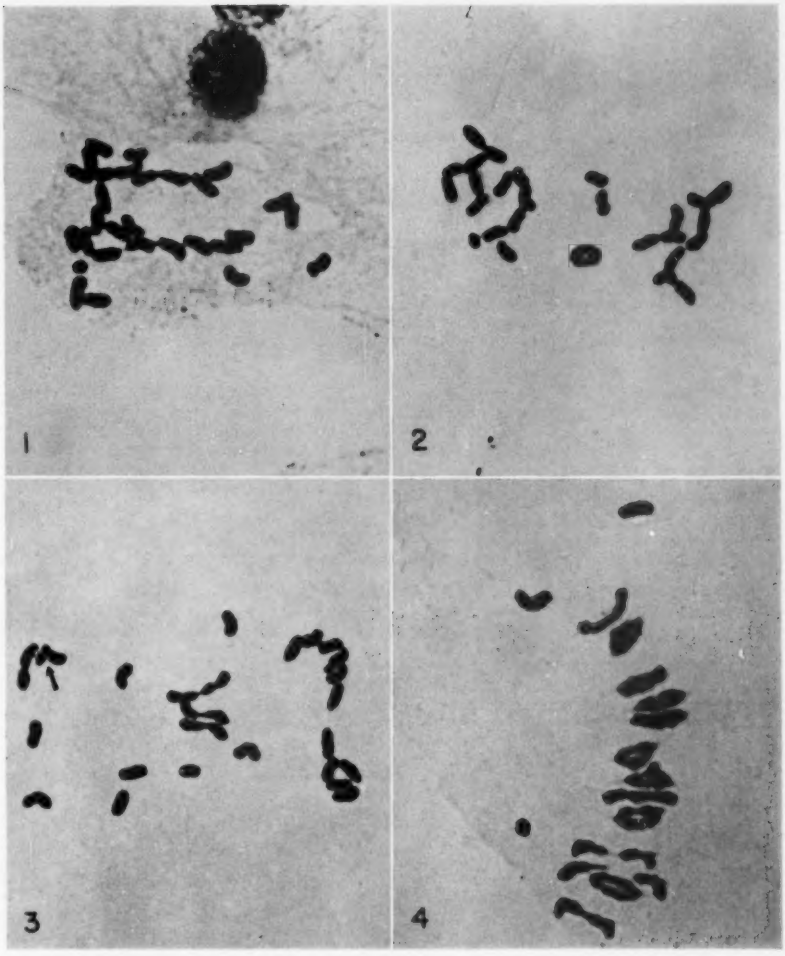


FIG. 1. Metaphase I cell showing 29 univalents.
FIG. 2. Metaphase I cell containing 1 closed bivalent and 27 univalents.
FIG. 3. Metaphase I cell exhibiting 3 bivalents and 23 univalents. Arrow points to the fragment.
FIG. 4. Normal metaphase I cell showing 13 bivalents, 2 univalents, and the fragment.

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# NOTES TO CONTRIBUTORS

## *Canadian Journal of Botany*

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